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BIOTECHNOLOGY AND THE DEVELOPING COUNTRIES: APPLICATIONS FOR THE PHARMACEUTICAL INDUSTRY AND AGRICULTURE*

Prepared by the UNIDO Secretariat

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EXPLANATORY NOTES

Mention of firm names and commercial products does not imply the endorsement of the United Nations Industrial Development Organization (UNIDO).

In addition to the common abbreviations, symbols and terms and those accepted by the International System of Units (SI), the following have been used in this study:

BGH	Bovine growth hormone
CGH	Chicken growth hormone
DNA	Deoxyribonucleic acid
E. coli	Escherichia coli
FMD	Foot-and-mouth disease
GRF	Growth releasing factor
HGH	Human growth hormone
MCA	Monoclonal antibody
Nif	Nitrogen fixation
PGH	Porcine growth hormone
R+D	Research and development
rDNA	Recombinant DNA
RIA	Radioimmuno assay
RNA	Ribonucleic acid

ORGANIZATIONS

EPA	United States Environmental Protection Agency
FDA	United States Food and Drug Administration
ICGEB	International Centre for Genetic Engineering and Biotechnology
INC	International Minerals and Chemical Corporation of Indiana
NIAID	United States National Institute of Allergy and Infectious Diseases
NIH	United States National Institutes of Health
OECD	Organization for Economic Co-operation and Development
OTA	United States Office of Technology Assessment
UNIDO	United Nations Industrial Development Organization
USDA	United States Department of Agriculture
WHO	World Health Organization

CONTENTS

	Page
EXPLANATORY NOTES	ii
INTRODUCTION	1 - 2
BIOTECHNOLOGY AND GENETIC ENGINEERING APPLICATIONS IN THE PHARMACEUTICAL INDUSTRY	3 - 28
- Recombinant DNA and the Pharmaceutical Industry	3 - 23
- Proteins	3 - 9
- Somatostatin	4
- Somatotropin	4
- Human insulin	5
- Interferons	5 - 7
- Interleukin-2	7 - 8
- Relaxin	8
- Thymosine alpha-1	8
- Brain opiates	8
- Urogastrone	8
- Growth Hormone-Releasing Factor (GRF)	9
- Vaccines	9 - 15
- Rabies vaccine	11
- Herpes vaccine	12
- Hepatitis B vaccine	12 - 13
- Cholera vaccine	13
- Typhoid vaccine	14
- Leprosy vaccine	14
- Malaria vaccine	14 - 15
- Antibiotics	15 - 18
- Monoclonal Antibodies (MCAs) and the Pharmaceutical Industry	18
- Diagnostic Kits	19 - 20
- MCAs and Cancer	20 - 21
- MCAs as Immunosuppressants	21

	Page
- MCAs for Passive Immunization	22 - 23
- New Biotechnology and Fermentation	23 - 24
- Discussion	24 - 28
BIOTECHNOLOGY AND AGRO-INDUSTRY	29 - 57
- Biotechnology and Animal Husbandry	29 - 37
- Prevention and Control of Animal Disease	29 - 33
- Animal vaccines	29 - 33
- Animal antibiotics	34
- Growth Promoters	34 - 35
- Improving the Genetic Composition in Animals	35
- Discussion	36 - 37
- Biotechnology and Plant Agriculture	38 - 45
- Genetic Improvements of Plants	38 - 42
- Phase I: Plant Cell Cultures	38 - 39
- Phase II: Genetic Engineering	39 - 40
- Phase III: Plant Generation	40 - 42
- Biotechnology and Pesticides	42 - 43
- Biological Control Agents	43 - 45
- Attractants	44
- Growth-affecting agents	45
- Storage survival	45
- Discussion	45 - 52
- Short-Term	46 - 49
- Medium-Term Projects	49 - 50
- Long-Term Projects	51 - 52
- Nif	51
- Photosynthesis	51
- Plant growth	51 - 52
- Conclusion	52 - 57

		Page
TABLE I	- ESTIMATED DEMAND FOR DRUGS BY THERAPEUTIC GROUPING VALUE ($ 000 MILLION)	58
TABLE II	- RELATIVE IMPORTANCE OF DEVELOPED AND DEVELOPING COUNTRIES AS DRUG MARKETS (1980-2000) ($ 000 MILLION)	58
TABLE III	- CHANGES IN THE LEADING 10 PHARMACEUTICAL MARKETS TO THE YEAR 2000 ($ 000 MILLION)	58
TABLE IV	- OTA ESTIMATES OF MONOCLONAL ANTIBODY MARKETS (IN MILLIONS OF 1981 DOLLARS)	59
TABLE V	- POTENTIAL OF GENETIC ENGINEERING FOR THE IMPROVEMENT OF VACCINES	60
TABLE VI	- INFECTIOUS DISEASES OCCURRING IN PLANTS	61
TABLE VII	- INFECTIOUS DISEASES OCCURRING IN HUMANS	62
TABLE VIII	- INFECTIOUS DISEASES OCCURRING IN ANIMALS	63
TABLE IX	- COST ESTIMATE FOR AN MCA LABORATORY	64
NOTES		65 - 73

INTRODUCTION

Throughout the developed world it is recognized that advances in biotechnology are likely to revolutionize wide sectors of industrial activity particularly those pertaining to drugs, fine chemicals, waste treatment, energy production and agriculture. However, there are those who suggest that the most important impacts of biotechnology will be on health and agriculture[1] and, in fact, developments in these areas during the last few years have been impressive. These two areas are used as a starting point for what will be a series of UNIDO papers relating the promises biotechnology holds for helping to solve the needs of developing countries.

There are two sections in this document. In the first, focus is on present and future activity in the pharmaceutical industry; the second similarly on agriculture. Each section begins with a description of products and processes which have appeared, or will do so in the short to medium term, in the area under consideration. In some cases, especially in the pharmaceutical section, it is possible to describe the major applications of the identified products and processes, to estimate the size of their actual or potential markets, to indicate the location of markets (whether in developed or developing countries), to note the costs of products to the consumer, and to compare the cost of the new products with products supplanted. When possible, additional information is provided on the research and development (R+D) that led to new products and processes. A small part of the pharmaceutical section is devoted to scale-up of fermentation processes involving genetically engineered organisms.

The descriptions of advances in the pharmaceutical industry and agriculture are followed by discussions of the relevance which described activities may have to developing countries. In particular, described activities are used as departure points for suggesting R+D which could be profitably undertaken by researchers in developing countries and at the proposed International Centre for Genetic Engineering and Biotechnology (ICGEB). Finally, a concluding section sums up the best approaches which may be taken by developing countries to realize applications from advances in biotechnology R+D pertaining to pharmaceuticals and agriculture.

Before proceeding, it is necessary to define that imprecise term "biotechnology" and to circumscribe the time frames in which advances take place.

The term "biotechnology" is often defined broadly; for example, the Organization for Economic Co-operation and Development (OECD) defines it as "the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services" (2). For the purpose of this paper, this definition is too broad; attention here is primarily focussed on "new" biotechnology which encompasses the use of novel biological processes such as recombinant DNA (rDNA), cell fusion, protoplast fusion and immobilized cells or enzymes for biochemical processes useful to agricultural, industrial and other applied practices (3). These novel techniques lay the basis for the revolution in biotechnology presently underway and as such are likely to have a major impact on development in the third world.

New biotechnology has gone through one phase of development and is now in the second. During what may be termed its first generation, issues pertaining to the conjectural hazards of rDNA research occupied much of the scientists' and knowledgeable public's attention. The risk issue was to a greater or lesser degree wrestled with in several developed nations. However, this period, approximately 1973-1980, need not be considered here. We are now in the second generation of biotechnology - the time when the field has matured sufficiently to allow for initial estimates of costs and benefits. Practical applications as a result of advances in the field are beginning to appear and a budding bioscience industry is emerging. This generation began in approximately 1978, continues through the present, and may not end for another two or three years. In the near future we shall move into the third generation of biotechnology; a period during which basic research will continue and accelerate and when impacts from applications of early research will be felt by large sectors of society. Additionally, a bioscience-based industry will become firmly established and other industrial sectors will use the new techniques for their own purposes. It is probable national economies, first in developed nations, and then across a spectrum of nations will become significantly affected by the new field.

BIOTECHNOLOGY AND GENETIC ENGINEERING APPLICATIONS IN THE PHARMACEUTICAL INDUSTRY

The importance of the pharmaceutical market to the world economy can be realized by regarding the following figures. The world pharmaceutical market was estimated at $76.3 billion in 1981. The market's growth rate is high, an estimated 10-12 per cent per year in the United States (US), 8 per cent in Japan and 5 per cent in the Federal Republic of Germany (FRG) (4). The world market for pharmaceuticals by biotechnology has been estimated to reach $5-10 billion in the year 2000 (5).

For the purpose of this paper, it is useful to divide pharmaceutical products into two broad groups; products that have been realized as a result of rDNA techniques and those resulting from cell fusion, ie, monoclonal antibodies. A third, short section has as its focus the fermentation processes whereby many bioproducts are realized.

Recombinant DNA and the Pharmaceutical Industry

The major types of pharmaceutical products which have resulted or will result in the short-term, from R+D using rDNA are proteins (including peptides, polypeptides and hormones), vaccines and antibiotics.

Proteins.

The basic building blocks of protein are the 20 naturally occurring amino acids. Two or more linked amino acids are termed peptides while several peptides joined together form polypeptides. Polypeptides join in an organized manner to make up protein.

The DNA of the gene controls RNA which assemble amino acids in the correct sequence and links them with peptide bonds to manufacture peptides, polypeptides and proteins. A single gene may code for the production of a peptide while the production of more complicated proteins may be controlled by several genes.

Some peptides have been marketed for drug use for longer periods of time including insulin and growth hormone. However, in the last ten years or so, over 30 new peptides have been discovered. Several have powerful regulatory functions in the body in, for example, controlling the secretion of hormones and regulating the transmission of neural impulses.

Analysts believe that research may eventually give rise to 50 or more peptides which will find use as drugs (6). Most of these are likely to command small, specialized markets worth perhaps $ 30 million per year, but a few, including an anti-arthritic peptide now under development, may command a market of perhaps $ 500 million per year.(6).

Several new and/or unique peptides and polypeptides have recently become available to clinicians as a result of rDNA research. At times, the situation is one where a product, not previously available, is looking for applications and markets. Nevertheless, we can be certain that as a result of genetic engineering applications heretoforth rare or non-available substances will appear with regularity. The following is a sample of the proteins which have appeared or are likely to appear in the short term:

- Somatostatin was one of the earliest proteins to be first synthesized, then cloned in the bacterium Escherichia coli (E. coli) (7). Its main action is to inhibit growth hormone secretion but it also affects a wide range of other hormones. It probably will have an important role in the treatment of endocrine, gastrointestinal and neurophyschiatric disorders (8). However, since adequate quantities for clinical purposes have not been available, definitive information about clinical uses will have to await results from presently underway clinical trials.

- Somatotropin, or human growth hormone (HGH), has been cloned in E. coli (9) and is now produced in sufficiently large quantities for clinical trials (10). The primary role of HGH is to control skeletal and cartilage growth, therefore, it is useful for treating hypopituitary dwarfism. Additionally, it may also find therapeutic use in treating disorders such as bone fractures, skin burns and bleeding ulcers. Osaka University is producing HGH using genetically engineered E. coli and is prepared to start clinical trials in late 1983 (11).

- Human insulin is the first rDNA-produced protein to be marketed. Basic research which eventually led to this product began in 1976 at the City of Hope, California; developmental work was done by Genentech; and scale-up and marketing by Eli Lilli and Co. Market approval by the US Food and Drug Administration (FDA) was granted in November 1982 for the product called Humulin. Average daily patient cost is estimated at $0.5 - 0.55 compared to $0.28 - 0.35 for mixed beef and pig insulin and $0.44 - 0.52 for purified pig insulin (12). Of an estimated 200 million diabetics in the world, approximately 10% are insulin dependent. The world market for insulin is approximately $400 million; it is 80% controlled by NOVO Industrie A/S (Denmark) and Lilly (13). As more companies develop the means to produce human insulin by using genetically engineered organisms, the price of the product could drop below that charged for insulin extracted from the pancreases of cattle and pigs.

- Interferons (14): Since interferon was discovered in 1957, it has become known that there is no single interferon, rather, at least three major types exist. Alpha interferon is produced by leukocytes (white blood cells); beta interferon by fibroblasts (connective tissue cells); and gamma interferon by T-lymphocytes (a type of white blood cell). Each type has numerous subtypes, for example, alpha interferon has ten known subtypes.

  Until recently, the only method for obtaining interferon was to collect large quantities of human blood, separate out the white blood cells and extract interferon from these cells. The process is exceedingly laborious and expensive; to extract approximately 50 milligrams of impure interferon from white blood cells costs close to $ 2 million.

  A second method now in use involves growing human cells in tissue cultures and infecting them with virus. The virus induces the cells to produce interferons which are then extracted and purified from the mixture of cells, substrate and viruses - at times, an expensive and difficult process. A beta interferon produced in this manner by the German firm Bioferon in April 1983 became the first interferon to gain governmental approval for use as a human drug to treat herpes zoster (15).

Though the cost of treatment per patient is not known it must be very large if one considers what is known pertaining to production and treatment: The Bioferon process yields up to 50 units of interferon per milliliter of culture. About 55% of the interferon is lost in the purification process. Each patient needs 500,000 units per day per kilogram of body weight and the treatment continues for three to five days (15). It can be seen that a 70 kg man would need between 10.5 million and 17.5 million units for a course of treatment. In order to produce that amount of interferon, between 767 and 778 liters of culture would have to be processed - a large quantity by any measure.

The third source of interferon is from bacteria which has been genetically engineered to produce alpha or beta interferon. This method has the greatest potential for scale-up in order to mass produce interferons (15). The US Office of Technology Assessment (OTA) estimates that the cost of interferon produced in this manner will be between $1 - 10 per million units compared to $50 per million units for alpha interferon extracted from white blood cells and $43 - 200 per million units for fibroblast produced interferon (16).

Sufficient clinical evidence is not as yet available to fully assess the extent of therapeutic applications of interferons (17). Some success has been seen with interferon in viral infections such as chronic hepatitis, colds, herpes simplex, herpes zoster and warts (18). In addition, there is evidence that interferons can enhance or inhibit cells that make up the immune system and can cause cells to stop growing. Therefore, these agents hold promise for the treatment of cancers (19).

Due to the promise which interferons hold for treating viral diseases and cancers, bioscience and pharmaceutical firms are making major commitments to manufacture these agents. For example, Biogene has developed rDNA processes to produce alpha and gamma interferons. Biogene's alpha interferon is currently undergoing clinical trials and if these prove safe and effective may get approval by the FDA for general use in 1984. Biogene (Sweden) has just begun human clinical trials on gamma interferon to test its effectiveness as an anti-cancer agent. Trials may cost $ 50 million with results forthcoming in four years (20). The US company Schering-Plough, which holds a 15% interest in Biogene, is constructing a plant for $ 106 million in Ireland to produce alpha interferon (21). Production is to begin in 1985.

In addition to Biogene, Genentech (US) is using an engineered E. coli to produce gamma interferon with a claimed yield of 5 million units per milliliter (ml) of substrate. More recently, Kyowa Hakko Kogyo (Japan) has devised an improved method for producing 10 million units per ml. It is claimed the latter company can produce sufficient gamma interferon for 5,000 cancer patients in a 200 liter batch of E. coli-containing medium after only a 2-3 day fermentation period (22). Nevertheless, as mentioned, Biogene appears likely to be in the lead for seeking clinical applications for gamma interferon.

The impact which interferons may come to have on the status of global health is unclear since it is not yet known if these agents are truly effective antiviral, anti-cancer agents. If they prove to be one or both, the impact is likely to be large. Preliminary indications are that interferons are effective against several viruses and certain cancers. However, negative side effects have accompanied interferon treatment (23).

The market value of interferons was estimated at $ 50 million in 1981 (24) but if they turn out to be effective anti-viral agents, the market may reach approximately the same level as the antibiotic market, estimated at $ 8.25 billion in 1980 (25).

- Interleukin-2: This substance is produced by the body in extremely small quantities and has a role in stimulating the so-called T-cells of the immune system to kill tumour cells. Interleukin-2 may thus prove to be a potent anti-cancer agent and could also have a role in stimulating depressed immune systems.

The most advanced work on Interleukin-2 appears to be carried out in Europe at the Laboratory of Clinical Pathology in Villingen (FRG) and in the Far East at the Tokyo Cancer Research Institute and the Ajinomoto Company (though teams in the USA and Belgium at both private and public facilities are not far behind). The T. Taniguchi group in Tokyo is possibly closest to being able to scale-up production of Interleukin-2 (26). However, Takeda Chemical Industries claim to have inserted Interleukin-2 genes into E. coli and are ready to industrialize the process (27).

- When this happens, the present Interleukin-2 market of $ 1 million is likely to expand manifold. The ultimate market cannot be predicted since the substance's degree of effectiveness as an anti-cancer agent is not yet known.

- Relaxin: This peptide hormone, secreted by the ovaries, helps ease the birth process by softening connective tissues. The agent may be useful in aiding childbirth and in treating rheumatoid arthritis.

  The gene coding for the production of Relaxin has been cloned in E. coli by a group headed by H. Niall at the University of Melbourne, Australia. Genentech has the exclusive rights to develop the product (28). Since the product is new and its effectiveness and safety is not known, it is impossible to predict its market potential.

- Thymosine alpha-1 is a peptide produced by the thymus gland which has important functions in the immune system (29). It has been synthesized then cloned in E. coli. It may have important therapeutic uses in immunodeficient individuals.

- Brain opiates: The first opiate called alpha endorphin was isolated by a team led by R. Guillemin (1977 Nobelist) at the Salk Institute. A bit later, several research teams almost simultaneously found a second group of opiates named enkephalins. The opiates have been found to effect the human response to stress, to act on pain, and to have a part in the control of obesity. They may also act on neurotransmitters (30). Within a short time, researchers will have elucidated the chemical structures of opiates and by working backwards will be able to construct synthetic genes coding for their production. Insertion of the genes into proper hosts will probably lead to large quantities of opiates becoming available for wide ranging research.

- Urogastrone is a peptide which acts to inhibit gastric acid secretion and it may, therefore, have use in controlling ulcers. Researchers at C.G. Searle and Co. have constructed a synthetic gene which codes for its production. Clinical testing is to have begun in early 1983 (31). Very little is known about this product due to its proprietary nature.

- Growth Hormone-Releasing Factor (GRF): In the summer of 1982 a person was found with a rare tumour which produced relatively large amounts of GRF protein. Researchers at the Salk Institute quickly determined its chemical structure and soon were able to synthesize GRF (32). Two months after these findings were reported, the small bioscience firm Creative BioMolecules in California had synthesized the gene coding for GRF and it is now available for purchase; for $ 50,000 the buyer obtains 10 micrograms of plasmids containing the gene and a protocol which tells how to use the plasmids (33). GRF appears to have an important role in controlling the amounts of growth hormone released by the pituitary gland.

Vaccines (34).

When a foreign body is introduced into an animal's body, the victim's immunological defense system reacts in several ways to neutralize the invader. If the invader is a microorganism such as bacteria, viruses, yeasts or other parasites, the victim's immune system can produce antibodies against different chemical structures which constitute the microorganism such as the surface proteins. The chemical structures eliciting an antibody response are called antigens. In an immune host, the antibodies attach to the antigens located on the invader's surface, thereby making it impossible for the invader to attach itself to the victim's tissue cells. Without the ability for attachment the invader cannot cause the damage which results in disease.

Vaccines are used for active immunization. These work by stimulating the recipient's immune system to produce antibodies against an invader but without causing disease. Though the antibodies produced survive only a short time, the cells which make the antibodies, the so-called B-cells, in co-operation with so-called memory cells, retain the ability to quickly produce the same type of antibodies for a future time when the host is again attacked. This ability confers immunity on the host which can last from months to years to a life-time, depending on the quality of antigenic response.

Passive vaccines consisting of sera or immunoglobins are used when there is no time for active immunity to develop. For example, if a person is bitten by a rabid animal, antirabies hyperimmune serum is administered as soon as possible to prevent the disease from occurring. Passive immunization does not confer longer term immunity.

Vaccines produced through the use of "classical" techniques commonly fall into one of two categories. First, a pathogenic organism can be grown in large quantities, then inactivated or killed by heat or chemicals. When injected into a recipient, the inactivated form stimulates antibody production without causing disease. A second approach is to weaken (or attenuate) a pathogenic organism by breeding it in the laboratory for numerous generations, thereby making it impossible for the organism to revert to its original virulent form. The attenuated live organism, when injected into a host, will stimulate antibody production without causing harm.

The new biotechnology techniques greatly extend the possibilities for the manufacture of safe and efficient vaccines. The following methods are samples of these possibilities:

1. Recombinant DNA techniques can be used to "snip" out the disease-producing genes from a pathogenic agent. After this has been done, the organism will in effect be attenuated and can be used as a live vaccine.

2. It is possible to identify those antigens of a microbe which are important in its pathogenecity (the so-called "protective antigens"). The genes coding for the production of these proteins can be inserted and cloned in E. coli thus making available large quantities of the desired protein. The protein, when injected into a host, acts as an antigen thereby stimulating antibody production.

3. Many bacterial species have protrusions on their surfaces called pili. Pili aid the organism to adhere to tissue cells. Pili can be extracted from a bacterial culture and can be used as an antigen in the manufacture of vaccine.

4. With the help of computers, it is possible to determine the chemical make-up and structure of antigens. Based on this information, it is possible to synthesize antigens in the laboratory and to use them as a basis for vaccine.

5. Genes coding for antigens may be removed from a pathogen and be inserted into a known non-pathogenic organism. When injected as a vaccine, the recipient's immune system forms antibodies against the non-pathogen and, at the same time, other antibodies will be formed against the foreign antigen coded for by the inserted genes. In particular,

researchers have found live vaccinia virus (used in smallpox vaccines) a suitable host for foreign genes from pathogens. Further, the vaccinia virus allows for the combining within itself of as many as six different viral genes, thus offering the possibility of vaccination against up to six diseases at one shot (35).

The market for human vaccines has stagnated in recent years in developed countries. At the same time, demand by developing countries has not risen, possibly because vaccines are expensive, difficult to store properly and supply lines tend to be weak or non-existent. The human vaccine industry was estimated at $ 95 million in 1980, but may go up to $ 275 million in 1985 as new vaccines reach the market (36). The animal vaccine market is larger (see below, page 30) Vaccines against viruses, bacteria and parasites will now be considered.

- Rabies vaccine: E. Ylverton and her co-workers at Genentech have been able to transfer the genes coding for the production of the rabies virus' glycoprotein coat to E. coli (37). This could be the first step towards the production of a recombinant live rabies virus vaccine since this glycoprotein stimulates antibody production in infected animals. Though an effective vaccine using inactivated viruses is available for humans a safe live virus vaccine is needed to eliminate the disease in wild animal reservoires (38) (see page 31 below).

  In addition to the Ylverton group at Genentech, a group at Transgene (France) in co-operation with the Wistar Institute (US) have reportedly reached the "testing" stage with a rabies vaccine developed by using rDNA techniques (39). Transgene has a development contract with the French vaccine manufacturer Merieux Institute.

  It is probable that a live virus vaccine against rabies will be widely available in approximately two years. Though the individual dose price is likely to be low, the many doses needed will add up to a huge cost which may have to be absorbed by the developing areas of the world.

- Herpes vaccine: An epidemic, perhaps pandemic, of genital herpes is now occurring in the US and other countries. Since herpes is not a reportable disease in most of the world, the true extent of this calamity is not known. In the US there are an estimated 5 - 20 million cases and the number grows by over 300,000 per year (40). Until an effective vaccine can be developed and used, the disease will continue to spread and the loss to society has been, and will continue to be very high. The market value of a vaccine against herpes is going to be high, an estimated $ 20 - 50 million per year (41).

  Researchers at Molecular Genetics (US) have cloned the genes coding for the herpes virus surface protein. The antigen produced has been tested in animals and appears to stimulate production of antibodies against the virus. Product scale-up and clinical trials are to be undertaken by American Cyamid. Several years of R+D are needed before results indicating safety and efficiency of the vaccine will be available (42).

- Hepatitis B vaccine: A sub-unit hepatitis B virus vaccine made by Merck and named Heptavax B has been available in the US since June 1982. The high price of over $ 100 has discouraged its use (43). Merck expects to eventually replace it by a vaccine made from bacterially produced hepatitis B antigen (44). Workers at Japan's Osaka University and at the University of California, San Francisco have cloned hepatitis B antigens in yeast cells that may become the basis for a vaccine. But the most promising approach may have been taken by a group at the US National Institute of Allergy and Infectious Diseases (NIAID) which inserted the gene coding for hepatitis B virus surface antigen into a vaccinia virus. When the genetically engineered vaccinia virus was used to inoculate rabbits, a high titre of antibody against hepatitis B was produced (45). This method has the potential of allowing the production of antigens against other disease-causing viruses. There is also a good possibility that in the near term researchers will be able to insert genes coding for two or more different antigens into vaccinia and other viruses.

Except for small populations at high risk, hepatitis B is not a major public health problem in the developed world. However, approximately 200 million people located mainly in developing countries are chronically infected with this disease and a large number die each year. Additionally, hepatitis may trigger a series of events which leads to cancer of the liver. The cost of caring for those afflicted by hepatitis or liver cancer is a serious financial burden, much of it borne by developing countries. It is suggested that the immunization approach using recombined vaccinia could duplicate the successful programme which eliminated smallpox (46). The implementation of this technology would only need a minimal investment since the facilities and expertise developed in the global smallpox eradication programme are still largely in place and are thus available.

- Cholera vaccine: A vaccine made up of inactivated cholera bacteria exists, but it is only approximately 50% effective and protects for a maximum of six months to twelve months. Recently researchers at the London School of Hygiene and Tropical Medicine, in work funded by the Wellcome Foundation and the UK Medical Research Council, have succeeded in isolating the gene coding for a toxin which is responsible for cholera's damaging action (47). After removing the toxin gene, researchers have bred the harmless bacterium in hopes of developing it to be the basis of a live vaccine. It is expected that the live vaccine, after being taken by mouth, will provide immunity lasting for three years. Work along similar lines is also proceeding at Italy's Institute Superiore di Sanita (48).

With rare exceptions, cholera affects the developing countries. It strikes many thousands of people per year, and causes incalculable damage in terms of suffering and medical costs. A safe and effective cholera vaccine would undoubtedly have a large market.

- Typhoid vaccine: A live vaccine against typhoid which is up to 96% efficient and is exceedingly safe (49) has been developed by a group headed by R. Germanier at the Swiss Serum and Vaccine Institute. No new biotechnology techniques were used, rather, a stable mutant lacking an enzyme needed to produce disease is the basis of the vaccine. When swallowed, the mutant bacteria undergo four to five cell divisions and infect and penetrate the wall of the small intestine; but then they "self-destruct". Perhaps genetic engineering techniques will be used someday to make an even safer, more efficient vaccine, but in view of the availability of the present vaccine such a project has low priority. However, rDNA techniques could be used advantageously to splice genes coding for other antigens into the crippled typhoid organism (50). Thus, immunizing antigens of dysentary and cholera could be produced by the appropriately engineered typhoid organism which would stimulate antibody production thereby conferring immunity to those diseases.

- Leprosy vaccine: A five-year development programme has led to a new vaccine based on killed Mycobacterium leprae. The product was developed at the National Institute for Medical Research (London) by a team headed by R. Rees (51). Though no new biotechnology was used to produce the new vaccine, R. Curtiss at Alabama State University, in a project funded by the World Health Organization (WHO), is using rDNA techniques to produce M. leprae antigens. This process should yield a vaccine available for clinical trials in a few years.

  Leprosy is overwhelmingly a disease of tropical countries. An estimated 11 million cases exist throughout the world with 60% in Asia; India has an estimated 3.5 million cases (52). With increasing resistance by the causative organism to dapsone - the primary therapeutic agent - there is cause to worry about a resurgence in leprosy. A safe, effective vaccine could be most helpful for eliminating leprosy from the world.

- Malaria vaccine: Since malaria goes through three stages of development in the human body, it is a difficult organism to produce a vaccine against. The first stage occurs when sporozoites are injected into the blood stream by an infected mosquito. A team headed by Ruth and Victor Nussenzweig at New York University has cloned E. coli the malaria sporozoite surface antigen from a malaria which infects monkeys (53). The process is now being patented but a disagreement has arisen as to who owns the intellectual property rights. The

minority funder of the Nussenzweig's research, WHO, wishes to retain these rights so the discoveries from the research will be freely available to its member countries. The proposed manufacturer of the future sporozoite vaccine, Genentech, wanted an exclusive license (54). Recently, Genentech withdrew from further negotiations so the manufacture of the future vaccine is in doubt (55).

The second stage of malaria is when the sporozoites reach the liver and there develop into merozoites which, in turn, are released into the blood stream and cause the symptoms of malaria. L. Miller directs a team at the US National Institutes of Health (NIH) that is attempting to develop a merozoite vaccine. However, this effort is at least two years behind Nussenzweig's research (54). Similar work is also under way at the Wellcome Laboratories in the UK where a team under R. Freeman has isolated a merozoite antigen with the ability to protect mice from malaria (56).

The third stage takes place when merozoites develop into gametes that are taken up by the mosquito when it bites an infected host. Theoretically, a vaccine could be produced against gametes but it is not known if such work is being done.

An effective vaccine against malaria will probably have to be active against both the sporozoites and the merozoites. It is possible such a vaccine will be available within three years. A vaccine is desperately needed because of growing resistance; the mosquito vectors have developed resistance against DDT and other insecticides and the malaria parasites against chloroquine. For these reasons, malaria is experiencing a resurgence throughout the world: an estimated 200 million people are now infected, out of which 2 million die per year (55).

Antibiotics.

In 1980 an estimated 25,000 tons of antibiotics was produced world-wide, mostly to treat bacterial diseases in man and animals and for use as animal feed additives. Of this amount, approximately 17,000 tons was penicillin, 5,000 tons tetracyclines, 1,200 tons cephalosporins and 800 tons erythromycins (57). The estimated value of the antibiotic market in 1980 was $ 8.25 billion (25).

Antibiotics belong to a group of biochemicals called secondary metabolites. Over 7,000 naturally occurring and more than 30,000 semi-synthetic antibiotics have been discovered since the early 1970s (58). Of these, approximately 150 are being produced but only about ten on a large scale.

Since the early 1950s, when penicillin began to be manufactured in large quantities, biotechnologists have been able to increase the yield of the antibiotic by quantum leaps using conventional methods of random mutation and selection. For example, the initial yield of penicillin was 10 units per ml but yields now can reach over 150,000 units per ml.

The genetic engineering techniques of protoplast fusion and rDNA hold particular promise for increasing antibiotic production. Protoplast fusion is a technique whereby the cell wall is stripped away from a cell but it leaves the cell's protoplasm intact. The treated cell is brought together with a similarly treated cell from another species and the two are allowed to fuse. The resultant hybrid cell will manifest genetic characteristics of both species.

Protoplast fusion has been used to create new antibiotics. For example, when Streptomyces griseus, which produces streptomycin, was fused with S. tenji-mariensis, a producer of istamycen, a hybrid was created capable of producing a new metabolite with differing properties (59).

Researchers at Bristol-Myers have used protoplast fusion to eliminate an undesirable side-product. Formerly, the industrial transformation of penicillin to cephalosporins resulted in 8 - 10% of the end product consisting of p-hydroxpenicillin - a substance which interferes substantially with the reaction. Through the use of protoplast fusion and selection, a hybrid strain has been developed which produces only about 0.5% p-hydroxypenicillin (60). Similarly, at Glaxo Laboratories the application of protoplasm fusion resulted in a strain which produces 40% more cephalosporin C than the previous strain (61). And at Pfizer, improvements utilizing fusion with other techniques have brought the production cost of oxytetracycline down to a few dollars a pound.

The use of rDNA is still more or less limited to research efforts. However, as Vournakis and Elander (58) have written, the promises to industry are great. Recombinant DNA can be used to insert genes coding for desirable enzymes into antibiotic producing organisms thereby adding one or two steps to biosynthetic pathways and leading to more efficient production. The transfer of acyltransferase genes may help in the production of cephalosporins more easily extractable from reaction mixtures. Much research is being done using rDNA on streptomyces (producer of 60% of the known antibiotics) to clarify

biosynthetic pathways and the molecular mechanism controlling gene expression resulting in the production of antibiotics. Such research is certain to lead to strain improvements of industrial significance.

However, one project using rDNA holds possibilities for an industrial pay-off in the short term. A co-operative endeavour between the Technical University of Braunschweig (FRG) and the Gesellschaft für Biotechnologische Forschung has resulted in the development of a strain of E. coli which is a super-producer of the enzyme penicillin acylase. The next step is to similarly design and produce an E. coli containing the gene for cephalosporin acylase. Such an engineered E. coli would be capable of converting the cheap precursor cephalosporin C directly into 7-aminocephalosporanic acid (7ADCA). Most of the different types of cephalosporins are derived from 7ADCA. Therefore, if this substance could be produced cheaply though the use of an engineered E. coli the now relatively expensive cephalosporins could be manufactured as inexpensively as penicillin (62).

Antibiotics are the largest therapeutic group in terms of sales among pharmaceuticals and this dominance is expected to continue. The estimated sales value of antibiotics is expected to reach $ 40.5 billion in the year 2000 (25). At the same time, it is expected that the developing world will face large shortfalls in antibiotic production; for example, UNIDO has estimated the Central American subregion will have a projected shortfall by 1986 of 1707 tons per annum (63).

Biotechnology R+D pertaining to antibiotic producing microorganisms seems to offer fine opportunities for researchers in developing countries and at the future ICGEB. The third world needs greater amounts of antibiotics, both to treat bacterial infections and for use as feed supplements in animal husbandry. Yet, only a few developing countries are in a position to undertake such research since few antibiotic R+D and production facilities exist in the developing world. For these select countries it would be most useful to familiarize their researchers with the techniques of new biotechnology, especially protoplast fusion. Antibiotic-producing developing nations such as Brazil, India, Mexico and Thailand would thus be able to increase yields and, perhaps, utilize cheaper or more readily available substrates in their antibiotic industry. Furthermore, their researchers would have the possibility of advancing to the forefront of antibiotic production technologies and remain there as the field progresses.

It is likely R+D employing protoplast fusion can result in short-term gains. Results from R+D using rDNA techniques seem to be more distant; at the present time only one project is known that seemingly offers pay-offs in the near term. At the same time, rDNA technique is not so different whether it is used to improve an antibiotic producing strain of microorganism or to engineer another to produce human insulin. Therefore, it is most useful for researchers in the biosciences, wherever they are located, to become acquainted with this powerful technique, since this knowledge can subsequently be used for whatever projects are decided upon by governments or institutes.

Monoclonal Antibodies (MCAs) and the Pharmaceutical Industry.

Antibodies have been used in the clinical laboratory since the early 1900s in immunologic essays to identify and quantify bacterial and viral antigens, drugs, immunoglobulins, and to type blood. Conventional antibodies used in essays are manufactured by injecting antigen into an animal. After sufficient time has passed to allow the animal's immune system to produce the maximum level of antibodies against the injected antigen, the animal is bled and the serum portion of the blood containing the desired antibody is separated and collected. As can be imagined, the quantity and quality of the product can vary considerably, not only from animal to animal but also from one bleeding to the next in the same animal. For these reasons it has been difficult to standardize reagents and to collect adequate amounts of reference antisera.

With the advent of MCAs, many of the shortcomings presented above have been overcome. MCAs are a recent development; in 1975 C. Milstein and G. Köhler working at UK's Medical Research Council were able to fuse a cell which produces an antibody but will not grow in a laboratory tissue culture with another which grows well under laboratory conditions (64). After fusion, the hybrid cell (or hybridoma) is able to divide and multiply to give rise to clones (identical progeny cells) which produce the required, single antibody (the MCA) (65).

Due to the recent discovery of MCAs, applications are just beginning to be realized. However, it is clear the number of applications from MCAs will be virtually unlimited. MCAs are likely to find major commercial applications in detection kits, to treat cancer, in immunosuppression and as specific immunoglobins.

Diagnostic Kits.

MCAs will quickly replace conventionally produced antibodies in immunologic essays. MCAs are already in use as cell surface markers particularly for typing white blood cells in order to aid in the diagnosis of leukemia, lymphomas and other diseases. UK's Celltech has released MCAs for use in typing the main human blood types. The world market for blood reagents is not so large, perhaps $ 15 million (66), but the early marketing of these MCAs may provide Celltech with a lead for marketing other diagnostic kits. The advantages of Celltech's typing MCAs over the conventional typing sera are: high degree of reliability from batch to batch, and the saving of blood so it can be fully used in transfusions and for making essential blood products.

As experience with MCAs grows, more diseases will be diagnosed earlier than possible heretoforth and with a greater degree of reliability. The number of possibilities for using MCAs to detect diseases is too large for a complete list to be provided here; it is sufficient to point out that in a recent issue of Telegen (67) work with the following MCAs was described: anti-allergen, anti-arthritis, anti-B cell, anti-DNA, anti-erythropoietin, anti-hepatitis B, anti-Hodgkin's disease, anti-leukemia, anti-melanoma, anti-neutrophil, anti-T cell, and anti-thyroid.

Another application for MCA detection kits is to help identify bacteria, viruses and parasites earlier and with greater accuracy than has been possible, thereby aiding clinicians in diagnosing infectious diseases. For example, R.C. Nowinski and his colleagues at the University of Washington Medical School have manufactured MCAs against three common sexually transmitted disease organisms: Neisseria gonorrhoeae, Chlamydia trachomatis, and herpes simplex virus types 1 and 2 (68). The use of MCAs improved the researchers' ability to detect each of the three organisms; particular advantage was gained in the early detection of Chlamydia. Instead of 72 hours being required for "routine quantitation" of this organism the use of the MCA allowed for an accurate counting in 18 - 24 hours. Even better, in approximately 90% of the cases, it was possible to detect Chlamydia in direct smears from the urethra and cervix by using immunofluorescence microscopy with MCAs. This procedure takes just 30 minutes (69). Recently the FDA approved the 30-minute test for marketing by Genetic Systems and Syntex, both in the USA (70).

What may be the first diagnostic kit to come on the market is one designed to detect Chagas disease. The causative organism, Trypanosoma cruzi infects perhaps 8 million people in South and Central America but many times that number carry the antigen to the organism. Since the kit's MCA only reacts with antigens from the living parasite, it will now be possible to perform large-scale population screenings for the disease and thus differentiate true cases from carriers. The MCA was developed by a group led by G.B. Broun at the University of Technology of Compiègne (France) and the kit is manufactured by the University's commercial subsidiary Biosys. An order for 600,000 tests has reportedly been received from Argentina (71).

As before, only a sample of the work being done on using MCAs to detect microorganisms can be mentioned. The following MCAs against microorganisms have been discussed in Telegen (67): anti-Hemophilis influenza, anti-hepatitis B, anti-malaria, anti-mirococcus, anti-poliovirus, anti-Salmonella, anti-streptoccoccus, anti-tapeworm and anti-trypanosoma.

MCAs and Cancer.

Since MCAs can be useful in differentiating tissue cells, researchers have been trying to identify specific cells that are associated with particular cancers. Though no one has been able to identify one type of cell which specifies all cancers, MCAs have been produced against several types of tumour cells including those associated with cancers of the breast, colon, liver, ovary, etc. (72). Two uses may be envisioned for MCAs in this regard. First, MCAs may become useful in the early detection of cancer and, second, the response of individual patients to treatment may be monitored.

A cancer detection kit called Tumortec is now on the market consisting of an MCA which has been fused to a radioisotope. The manufacturer claims that trials indicate the kit allows certain cancers to be detected at an earlier stage than possible before. It is claimed that Tumortec detected 100% of genital tumours, 75% of primary lung tumours, and 50% of primary breast tumours. Tumortec R+D was done by the Lovelace Medical Foundation (Canada), the license holder for Canada, South America and Europe is Summa Medical while Nuclear Pharmacy is the licensee for the USA. The product is aimed at markets in developed countries with sales projected for 1983-1984 at $ 4.5 million and eventually rising to $ 300 - 400 million on a world-wide basis (73).

MCAs may also be directly useful for treating cancer. Two possible therapeutic approaches can be visualized. First, it is possible to construct MCAs against specific malignant cells, ie, against those which carry surface immunoglobulins. Such an approach was taken by clinical investigators who treated a patient, suffering from what was considered terminal lymphoma, with injections of MCAs produced against this person's malignant B cells. As a result, the patient's disease was put into remission for over one year (74). Similarly, Damon Biotech, in a joint programme with the National Cancer Institute, is attempting to make individualized MCAs for each cancer patient. The first type of cancer to be treated is B-cell lymphoma. Damon expects to be able to manufacture patient-specific MCAs in 8 - 10 weeks at a cost of $ 30,000 - $ 50,000 per patient per treatment course (75).

The second approach is to kill individual cancer cells without harming normal tissue cells. This is being done by coupling the tumour-specific MCA with a cyto-toxic agent. When the MCA connects with the targeted tumour cell the toxic agent is brought into contact with the cell's surface and thereby damages or kills the malignant cell. Several groups are trying this approach of cancer treatment but it is too early for results to have been published (76).

MCAs as Immunosuppressants.

Whenever someone has to undergo a major organ transplant, for example, a kidney transplant, it is necessary to immunosuppress that person. This is now done by administering a toxic drug which inhibits cell division. The tissues composed of rapidly dividing cells, particularly the bone marrow, are thus most affected by these agents. However, the cells which are most active in organ graft rejection are the T-cells, so anti-T-cell MCAs should work well in immunosuppression. Such MCAs have in fact been constructed and clinical trials are proceeding (77).

A related use for MCAs may be in the suppression of allergies. MCAs may be used to suppress immunoglobulin E which has a major part in determining an individual's response to allgergens (78). The market for such a product would be large since huge quantities of anti-allergens are sold in developed countries. The market value is difficult to estimate since anti-allergens, such as anti-histamines and steroids, have multi uses.

MCAs for Passive Immunization.

MCAs may be used for passive immunization and thereby serve to prevent disease by "capturing" targeted pathogens before they can cause mischief. One such MCA is already on the market for use to prevent scours (see below, page 32). Undoubtedly, MCAs will soon be developed to be similarly employed against human disease agents.

In a similar context, MCAs may serve to "capture" the agent it is targeted against so the "captured" organism can be further studied. For example, rDNA can be used to remove the genes coding for the surface antigen of a "captured" virus and those genes can be inserted into a bacterial host. Through cloning sufficient antigen can be produced to enable researchers to design and manufacture appropriate MCA against it. It is probable that specific immunoglobins could quickly be developed against those viruses that have been investigated thoroughly and whose surface antigens have been cloned, including the hepatitis A+B viruses, polio virus and rabies virus.

Rather than serving as therapeutics themselves, MCAs are likely to have a larger role in helping researchers to identify and separate out antigens capable of becoming the basis of a vaccine. Two lines of research using MCAs and having possible uses to developing countries will be mentioned. The first is the identification of antigens, useful for manufacturing a vaccine, carried by the malaria parasite Plasmodium falciparum. MCAs have been constructed against these antigens and are being used to isolate and recover individual targeted antigens so these, in turn, may be cloned and studied (79). In the second case, researchers have used a series of MCAs to clarify the disease process of Chagas disease. Their findings suggest that the degenerative changes in human tissues, which accompany infection with the causative organism Trypanosoma cruzi, may be largely due to an autoimmune phenomena (a condition resulting from the body's immune system attacking its own tissues). This finding was suggested after researchers discovered an MCA which reacts both with antigens on the parasite's surface and with surface molecules of affected tissues. Therefore, the antibodies which a host's immune system manufactures against the invading parasite would also attack the similar tissues, thereby causing the disease symptoms (80). The next step in research will be to isolate and recover the antigen (probably with a specific MCA) in order to clone it for further study.

A final example of MCAs as therapeutic agents concerns their use as antivenoms. Each year, many thousands of persons throughout the world are stung or bitten by venomous animals with a high concomitant loss of life and health. In some cases, antivenoms are available but they may not be effective and their use can cause allergic reactions. MCAs against quite a few bacterial toxins are in existence; there is reason to believe MCAs against most common insect and reptile toxins will also soon be available. A start has been made by researchers at Vanderbilt University Medical Centre (USA) who have developed an MCA aimed at an active enzyme in the venom of the brown recluse spider. To the 5,000 plus people hospitalized in the USA each year after having been bitten by the spider, the antivenom MCA will be far superior than conventional antibodies made from rabbit blood. Clinical tests are to have begun in 1983 (81).

New Biotechnology and Fermentation

Fermentation is the biochemical process whereby microbes convert raw materials, such as glucose, into a product such as a protein (82). The problems and promises of fermentation processes in relation to genetically engineered organisms has been reviewed by D.N. Bull (83); here a summary of his findings will be presented.

Inevitably, the results gained from R+D will have to be scaled-up to the point an industrially worthwhile production level is achieved. According to Bull, most of the fermentation problems with genetically engineered organisms are an extension of classical problems of industrial fermentation. However, special problems can arise with the use of certain genetically engineered organisms including the following three:

- Proteins now being produced by genetically engineered organisms are encoded for by genes carried by plasmids. Since plasmids are independent, self-replicating entities (though existing within a host cell), they may not replicate at the same pace as the host. If they replicate too fast, the host will be destroyed; if too slow, they will be diluted out. Therefore, fermentation conditions have to be regulated so the replication of host and plasmid is most nearly in phase.

- Heat transfer may present a difficult problem when newer fermentation processes take place at higher rates than is usual. Since fermentation processes are extremely heat sensitive, much more information is required about heat build-up and heat transfer during large-scale fermentation.

- Increased cell densities in newer fermentation processes have caused problems pertaining to mass transfer, especially oxygen transfer. For maximum cell growth, oxygen and other nutrients have to reach the cells in the fermentation mixture in optimal quantities while the amount of energy required to do so is minimized.

The overcoming of these and other problems is extremely important in order to manufacture a product able to hold its own as to quality and price in an extremely competitive market. The technological approaches Bull suggests, which could accomplish this aim are rather complicated and capital intensive. For example, several parameters can be continuously measured by sensitive sensors hooked up to a computer which continuously monitors the reaction and compares the generated data to a previously elaborated "ideal" model. Through a refined feedback control, nutrients are added and waste products disposed of on the command of the computer. Thus optimal conditions are maintained until the fermentation reaction is complete.

It can readily be seen that such a sophisticated control system when coupled to the requisite pilot plant would be very costly and would require highly trained technicians to run. An alternative approach could be to simplify fermentation processes, particularly in regard to industrially important genetically engineered organisms. Such a programme would be particularly appropriate within a broad, interdisciplinary programme as envisioned for the ICGEB. Only within a sizeable programme would it be possible to perform advanced R+D in reference to fermentation vis-a-vis genetically engineered organisms since scientists from a variety of disciplines would have to partake in addition to chemical and biochemical engineers. For this same reason, it is doubtful if more than a few developing countries could profitably involve their researchers in this type of R+D at the national level.

Discussion

The world drug market is huge - an estimated $ 75 billion in 1980 (84). Yet, it is to grow at an ever increasing rate through the year 2000 (see table I). Though the drug markets in developed countries will be quite a bit larger in 2000 than those in developing countries, the latter will by no means be insignificant (see Table II). A few developing countries are expected to become major pharmaceutical markets by 2000, in particular Brazil, Mexico and Argentina (see Table III). And, as has been mentioned, drugs manufactured through applications of new biotechnology techniques may

reach a value of $ 10 billion by this time.

So far, the market for MCAs has been rather small; an estimated $ 15 million in 1982 (85). However, this value is expected to rise at an exponential rate in the next ten years and to reach over $ 5 billion in 1992 (85). It is probable that MCAs for use in diagnostic kits, research reagents and drug delivery systems will be the fastest growing sector of biotechnology during the next ten years (see Tables IV and V).

Despite being aware how important the world drug market is in both financial terms and in terms of adding to the well-being of mankind, a conclusion must be made that it will be difficult, perhaps impossible, for the developing countries and the proposed ICGEB to become meaningfully involved with new drug formulation via biotechnology R+D. The main reasons are the very considerable investments which are needed to scale-up laboratory procedures and to perform the high-cost clinical trials necessary before a product can be sold.

The cost of the basic and applied research required to discover and develop a product to the scale-up phase is negligible when compared to scale-up and manufacturing costs. A few examples demonstrate this point. As mentioned, G.D. Searle is spending $ 106 million to build a plant to manufacture alpha interferon. The joint effort between Cetus and Shell Oil Company to develop and manufacture beta interferon has cost $ 25 million since 1980 and this figure will double before the product is marketable. The total of $ 50 million includes $ 10 million to build a new manufacturing plant (86). Lilly reportedly spent $ 60 million to set up a manufacturing plant for its rDNA insulin and the clinical trials for Humulin reportedly cost $ 12 - 20 million (87). On a more general level, Merck, one of the largest manufacturers of drugs, is now spending $ 338 million (11% of its sales) on R+D emphasizing new biotechnology and the neurosciences. Merck's Chairman, J. Heron, has stated that only large companies can afford the huge costs and long development time needed to bring a new drug on the market (88). Drug companies in the USA estimate that it takes 8 - 10 years and almost $ 100 million to bring a new drug to the market (87). This situation is not peculiar to the USA; for example, in Holland it takes an estimated ten years to develop a product from its initial synthesis to marketing and the process costs approximately $ 35 million (89). One possible exception to high development costs may be peptide drugs. Small firms specializing in peptides estimate the development costs at $ 5 - 10 million per drug (6).

In view of the long lead time and the high costs commonly associated with pharmaceutical R+D, countries may be forced to choose among the following development strategies:

1. Make whatever sacrifices necessary to achieve a previously agreed upon level of self-sufficiency in drug manufacture. This strategy might be chosen by a country such as Brazil, which has a large market for drugs and which has an adequate science and technology infrastructure to accomplish its aim. (Brazil, in addition, has apparently made a major commitment to become a world leader in the manufacture of tropical medicines (90).

2. Identify one, or a few, high priority R+D projects and concentrate resources to accomplish them including making certain resources are available for scale-up and manufacture. This option could be attractive to a country that has, for example, one or a few diseases that demand special attention, perhaps because the disease(s) in question is (are) very destructive in human and financial terms. If so, the country may decide to initiate or take special measures to alleviate the problem. These measures could be one, or a combination, of the following:

(a) Set up research groups, within the country's universities or other research institutes, directed to perform appropriate R+D in order to alleviate the problem. Resources should be available to make certain results from R+D can be applied for maximum effect. This option might necessitate the establishment of a basic research science infrastructure.

(b) The country can identify groups in advanced nations already performing pertinent R+D and some type of joint venture agreement could be set up between the two. Again, adequate development and scale-up funds would have to be available so research results can be applied effectively (see (c) also).

(c) The country could contract for needed R+D, using either bioscience firms in advanced nations or the proposed ICGEB. The contract could contain a proviso for transferring the technology and know-how required to apply results. The country would in this instance concentrate its efforts on making sure an adequate manufacturing infrastructure would exist to apply and commercialize from contracted research results.

(d) The country could request bioscience-based firms to bid for a contract to provide a certain quantity of finished drug designed to alleviate the identified problem. The country would itself market the finished product.

3. The country could wait and hope that some day an appropriate drug would appear on the world market. At that time, the country would purchase and dispense the drug.

From the information so far provided, it would seem that R+D pertaining to MCAs for use in diagnostic kits and as detection agents offers impressive opportunities for researchers in developing countries and at the future ICGEB. The reasons for this conclusion can be summarized as follows:

1. The technology necessary to produce MCAs can be mastered readily by bioscience researchers and technicians. Furthermore, the cost of designing and constructing MCAs is reasonable, especially when compared to the costs associated with designing and producing therapeutic agents.

2. Scale-up of MCA production is not a problem. The milligram amounts needed for research and diagnostic markets can be satisfied by the current two methods of producing MCAs, tissue culture or ascites production in primed mice (65).

3. The sizeable demand for MCAs in developing countries is likely to be for assays of infection agents, drugs and body fluid substances (for example, serum and blood levels of hormones). An adequate bank of desired MCAs for these purposes could be built up at a rapid pace. An added plus is that the present methods of assays in advanced countries are to a great extent based on radioimmunoessay (RIA) techniques. RIAs are characteristically time-consuming, technically demanding and are dependent on the availability of expensive equipment such as gamma counters and expensive reagents. For these reasons, RIAs are not widely available in the third world. Assays based on MCAs will probably supplant RIAs in the near future (91) because they are more specific, at least equally accurate, and are cheaper and easier to use. It can be seen that familiarity with MCAs and their uses could allow researchers and clinicians in developing countries to skip over RIAs and directly enter a MCA assay phase, thereby saving time, effort and money. Furthermore, third world scientists would be very quickly at the cutting edge of the technology.

4. Basic data about strains of disease organisms are in many cases lacking in developed countries and such a lack can hinder comprehensive efforts to control and eliminate particular diseases. The availability of appropriate MCAs could enable a country to perform useful epidemiological research in order to fill the information gaps in reference to indigenous diseases.

5. Often, one of several variants of a pathogen may cause a particular disease. Strains of bacteria can vary greatly as to their resistance to antibiotics and for this reason, effective treatment may be delayed because the wrong antibiotic is initially administered, thereby lengthening the time of incapacitation and raising the cost of treatment. In the case of viruses, vaccines produced against one virus variety may be ineffective when another variant actually strikes.

   In general one finds that early, effective treatment saves much suffering and money. As has been described, the use of MCAs as diagnostic tools can indeed help a clinician form early diagnosis and thereby allow him to initiate timely, correct therapy.

6. Familiarity gained from developing and producing MCAs for use as diagnostic aids and as detection agents can be most useful if a decision is subsequently made to develop MCAs as therapeutic agents. Knowledge and know-how gained furing the first phase can easily be applied in the second phase - the technology is the same.

7. Finally, the regulatory requirements to market diagnostic aids are less strict than those for therapeutic agents. In the USA, if a new diagnostic agent is similar to one previously approved, a "fast-track" approval process taking only 10 days, or less, can be taken. In case the diagnostic kit is completely new, the approval process rarely takes longer than one year. And, of course, costly clinical trials are not required. An indicator of how fast MCAs are penetrating markets is the fact that by early summer 1983, the FDA had approved over 20 clinical tests based on MCAs (92).

BIOTECHNOLOGY AND AGRO-INDUSTRY

M.S. Swaminathan has suggested that biotechnology will have its greatest impact on agriculture and health. Further, "a major agricultural asset of many developing countries is the vast, untapped production reservoir arising from the prevailing gap between potential and actual yields even at current levels of technology (93). In this section attention will be focussed on the possibilities which new biotechnology techniques offer to agriculture, especially in filling the gap Dr. Swaminathan identifies. For the sake of clarity agriculture is divided into two parts - animal husbandry and plant agriculture. As in the preceeding section, recent major advances in these areas will be first considered as well as likely near-term future developments. An assessment of recent and future advances, in turn provides a basis for suggesting possible avenues for R+D in developing countries and at the future ICGEB.

Biotechnology and Animal Husbandry

Productivity in animal husbandry can be raised by three methods; decreasing loss of animals due to disease, increasing yields by maximizing conversion of feeds to animal protein, and by improving the genetic composition of animals. (The related possibility of introducing a gene or genes into an animal's genome which would enable that animal to produce a valuable protein - a type of gene farming - will not be discussed here since its realization is likely to take a long time (94).

Prevention and Control of Animal Disease.

Direct prevention and control of animal diseases is best accomplished through the use of appropriate vaccines, and through the therapeutic use of anti-toxins and antibiotics. As will be seen, new biotechnology techniques have applications in each of these approaches.

Animal Vaccines.

The theory behind vaccine R+D and production as well as the application of biotechnology techniques to vaccine production were discussed above (pages 9 - 15). The potential of genetic engineering for the improvement of animal vaccines has been considered by Sir W. Henderson (see Table V).

Most animal diseases are not reportable, therefore, it is difficult to estimate their incidence rates. Nevertheless, the loss due to animal disease is very large, an estimated $ 50 billion worldwide (95). The animal vaccine market is also large, an estimated $ 1 billion in 1981 and the market is estimated to grow 20-25% per year between 1981 - 1985 (96).

In addition, the presence of infectious disease may cause serious but indirect losses, difficult to calculate. If diseases could be controlled, it is likely a substantial increase in animal supply would result. For example, bovine trypanosomiasis has been described "... economically as the most important disease of cattle on the African continent" and "... a major constraint to agricultural and socio-economic development in vast areas of Africa" (97).

As was mentioned above, it is possible to produce vaccines and sera against viruses, bacteria and parasites (such as protozoa) which are responsible for causing animal diseases.

Vaccines against viruses are the easiest to produce and are, therefore, the first to have been marketed. Examples include:

- Foot and Mouth Disease (FMD). This disease is a highly contagious, severely debilitating virus infection which affects cloven-hoofed animals. Since the 1960s, a killed virus vaccine has been available but, on occasion, it has been found unsafe. It is unstable and it does not confer long-lasting immunity. Despite these problems, in Europe more than one billion doses of FMD vaccine are sold per year at an estimated cost of $ 200 million (98). The disease is endemic in South America, Africa, much of Asia and in some European countries.

  A US Department of Agriculture (USDA) research group on Plum Island in New York found that one of the four proteins (VP3) which constitute the viral coat of FMD virus is an effective antigen (99). A collaborative effort between the USDA and Genentech has led to the development of a genetically engineered E. coli producing large quantities of VP3 antigen. It is claimed that two injections of the antigen in an oil adjuvant gives protection to cattle and swine and is completely safe. Field trials are

soon to commence in South America and a commercial vaccine could be ready by the mid 1980s (100). Scale-up of vaccine is to be undertaken by International Minerals and Chemical Corporation of Indiana (IMC). Other entities working on producing a genetically engineered vaccine are Biogene and Molecular Genetics in the USA, Pirbright Institute and the Wellcome Foundation in the UK, and Biotechnology General in Israel.

- Rabies: The rabies virus can attack most warm-blooded animals and is transmitted by the bite of an infected animal. The disease is prevalent in all temperate and tropical areas though the animal species most affected vary according to locale. Rabies is particularly costly to developing countries. For example, in South America damage to cattle from the bites of diseased vampire bats is estimated to cause a loss of $ 29 million per year (38).

  The most advanced work to produce a genetically engineered vaccine appears to be taking place at Genentech (101) and at Transgene (France). The latter company announced in 1982 the development of a genetically engineered vaccine which is to be tested by Institut Merieux (102).

- Blue Tongue: The disease is caused by insect-transmitted virus attacking mainly sheep; it is endemic in Africa and the USA. Researchers in Israel, South Africa and the USA are using genetic engineering techniques in an attempt to produce effective vaccines (103).

- Other viruses: Other viral diseases are being investigated but vaccines may be far off. Significant diseases on which research is proceeding are Marek's disease, Newcastle disease, African swine fever, canine parvovirus infection, Rift Valley fever, pseudorabies and African horse sickness.

Bacteria are genetically quite a bit more complicated than viruses and the state of anti-bacterial vaccine R+D reflects this fact. Two vaccines have been produced against a bacterial disease; two more may soon appear.

- Scours: This deadly disease is a neonatal calf diarrhoea caused by an enteropathogenic strain of E. coli. It is responsible for severe economic losses throughout the world.

A new-born calf or piglet receives immunological protection from a a thick secretion in the udder called colostrum. If the young animal is deprived of colostrum or if it has an absorbtion problem, it is likely to contract scours.

The enteropathogenic E. coli causing scours is notable because it produces adhesion factors that allow the bacterium to attach to the intestinal wall. As a result of work started in 1979 at the Dutch Institute of Health, researchers have been able to insert the genes coding for the adhesion factors K88 and K99 into a laboratory form of E. coli. Through cloning, sufficient quantities of the adhesion factors have been made available for use in vaccines. Developmental work was done by the Dutch company Intervet International, a subsidiary of the multi-national Akzo Pharma (98). Two different genetically engineered vaccines against scours were released by Intervet last year; the US companies Norden Laboratories and Cetus expect to market similar vaccines within three years.

The use of MCAs for passive immunization is described above (page 22). One such MCA has been developed by Molecular Genetics and has recently received approval from the Canadian Government to be used against scours. It is the first MCA approved by a country for disease prevention. US approval is also expected shortly (104).

- Furunculosis and Fish Vibriosis: The causative bacterium of furunculosis is Aeromonas salmonicida while Vibrio anguillarum causes vibriosis. No vaccine exists against the first; only a short-lived oral vaccine against the second.

The agricultural industry which produces fish at risk of attack by these organisms is worth in excess of $ 300 million per year in the USA. Estimates for the rest of the world are not available, but it is known aquaculture is important to many nations. Therefore, R+D being undertaken at the University of Washington to produce genetically engineered vaccines against the two fish pathogens can have wide impacts if successful. The worldwide fish vaccine market is now estimated at $ 8 - 14 million per year (105).

Parasites are even more complicated than bacteria and, therefore, present difficult problems to researchers seeking to produce vaccines against this group of organisms. One on-going project, at the University of Pennsylvania School of Veterinary Medicine, is aimed at producing a vaccine against canine filariasis. The research group has already produced an MCA filariasis detection kit, marketed by Mallinckrodt, and are producing MCAs against a variety of filaria antigens. A successful vaccine could win a $ 10 - 25 million per year market in the USA (106).

Another project is underway at the University of Illinois (funded by IMC) aimed at producing vaccines against bovine and canine babesiosis. The first is of extreme economical importance; an estimated 500 million cattle throughout the world are endangered by the causative organism Babesia bovis. An expert has stated that "Babesiosis is presently considered as one of the most important constraints in production of cattle in most regions with tropical and semi-tropical climates (107). M. Ristic, a vetinary scientist at Illinois, has isolated glycoproteins shed by the parasite when it enters the blood stream and these glycoproteins, when combined with the plant extract saponin, have proven to be the basis for an effective vaccine. The vaccine has passed first trials and field trials are now underway in Australia and Venezuela (108).

The relatively small number of references in the scientific literature to MCA R+D pertaining to animal diseases suggests that this area is lagging. In particular, R+D focussing on animal diseases of importance to developing countries (see Table VIII) may be seriously neglected. Yet, it should be made clear that all of the uses of MCAs directed towards human health (discussed on pages 18 and 23) are also applicable to animal health. For these reasons, MCA R+D of relevance to animal health appears to offer particular promise for short and medium-term gains to researchers in developing countries.

Animal antibiotics.

The same considerations apply to the manufacture of antibiotics for animal use as for human use (pages 15-17), so they need not be repeated.

Growth Promoters.

The production of HGH through the use of genetic engineering techniques was discussed above (page 4). Other types of growth hormones have also become available in industrial quantities and several companies are involved in their production. At present, in addition to HGH, Genentech is producing bovine and porcine growth hormones (BGH and PGH) and have licensed production of these substances to Monsanto (USA) and Kabi Gen (Sweden). More recently, applied Molecular Genetics (USA) has announced that its researchers have inserted the chicken growth hormone (CGH) gene into E. coli with resultant good yields of the hormone (109).

Research is beginning on the use of growth hormones as growth promoters in animals. A group at Cornell University have performed a number of short-term experiments which involve the administration of BGH to lactating cows. After 12 weeks, cows increased their production of milk by approximately 10% with no difference in feed intake (110). However, preliminary evidence indicates production increase might have taken place at the expense of a breakdown of body fat which could result in an eventual impairment of the animal's health.

Research on smaller animals is further along. A particularly exciting project using mice was recently reported. A team of scientists led by R. Palmiter of the University of Washington introduced into mice a foreign gene which codes for the synthesis of growth hormone (111). The genes proved to be functional and the treated mice responded to the secreted growth hormone by growing almost twice as large as untreated litter mates (112). These dramatic results were widely reported in the popular press usually with an accompanying striking photo of two ten-week-old mice side by side - one weighing 41.2 grams, the other 21.2 grams (113). Despite this remarkable achievement it is clear much research has to be done before applications to animal husbandry can be considered. Several problems have to be overcome. First, after the foreign gene has been inserted into an animal there is no way of controlling the production of growth hormone coded for by the gene. Therefore, no assessment can be made as to the ultimate effects of this procedure, especially in larger animals. Second, the techniques used by Palmiter, et. al. are extremely complicated with no short-term possibility for mass

application. Third, questions remain whether the animal protein would be tasteful, or even edible, after treatment; whether the treated animal would consume proportionally the same, or greater amounts of food; whether it would be as healthy as untreated mates, etc.

It is clear much research needs to be done before it is possible to enhance the natural growth mechanisms in animals to produce more animal protein. At the same time, the question has to be answered whether proteins from treated animals slaughtered for human consumption will be contaminated by unhealthy residues. For these reasons, long-term studies are required to establish the efficiency and safety of using growth promoters in animals. It will be at least ten years before meaningful information pertaining to larger animals can be generated; a little less time will be required for small animals such as chickens. For these reasons it appears as if the area of R+D pertaining to growth promoters should not have a high research priority to developing countries.

Improving the Genetic Composition in Animals.

Several breeding technologies are used throughout the world to improve the genetic composition of animals, including estrus synchronization, artificial insemination and embryo transfer. These technologies do not usually use new biotechnology techniques and are, therefore, outside the scope of this paper. However, recently an important development in cattle breeding using MCAs has been reported. Genetic Engineering, Inc. (USA) has produced an MCA against a male determinant found in cattle and by using this MCA, the sex of a six-day old embryo can be determined (114). With the new technique it has become possible to selectively implant female embryos into surrogate mothers; an important development for the dairy industry since a cow may be worth $ 2,500 while a male calf is worth perhaps $ 50. The application of this technique may also become important to developing countries. Dairy cows in developing countries typically produce approximately 1,360 kgs. of milk per year while a selectively bred US cow produces approximately 7,270 kgs. of milk (114). The new technique will enable cattle breeders in developing countries to purchase frozen embryos from highly productive cows and implant them into locally bred surrogate mothers. As a result, the fetus will inherit much of the mother's immunity to local diseases and therefore will have a better chance of adapting to the local environment after birth.

Discussion

Animal husbandry is extremely important to the well-being of mankind; animal products contribute over 56 million tons of high-quality protein to human food, equivalent to approximately 50% of the protein produced by all cereal crops (115). Yet, funds for R+D pertaining to animal husbandry has been less than 15% of the total devoted to agriculture (115). The pay-offs from R+D could be very great. For example, a 10% reduction in loss from animal diseases could add 2.4 million kgs. of protein to the world's food supply (116). Clearly, there are great opportunities for R+D in this area by researchers in developing countries and at the future ICGEB.

The animal vaccine market is large and is growing at a high rate. This area offers many R+D opportunities; more so than is the case of human vaccines because requirements are less exacting and the investment needed to bring a product to the market is lower. At the same time, a number of diseases which affect commercially valuable animals in developing countries are not targets for present-day vaccine R+D. Projects in vaccine development having the possibility for short-term pay-offs include research pertaining to all groups of pathogens. Particularly important viral diseases include African swine fever, bovine viral diarrhoea, goat catarrhal fever, hog cholera, Rift Valley fever, psendorabies, etc. Bacterial diseases include brucellosis, contagious bovine pleuropneumonia, infectious keratoconjunctivitis, leptospirosis, mastitis and tuberculosis. Parasitic diseases include anaplasmosis, East Coast fever and, especially, trypanosomiasis.

There are many possibilities which MCAs offer to human health; a similar situation exists as to animal health. The latter is possibly one of the more neglected areas of R+D. Since so many opportunities exist, only a sampling can here be presented.

A major problem facing breeders, alluded to above, is that of transferring high-performing animal breeds from the USA and Europe to tropical developing countries. So far this has proven impossible, unless extraordinary protective measures are taken, because local diseases will decimate the imports. Use of MCAs may provide the tool for analyzing the components of animal immune systems which provide protection to local pathogens. Other MCAs can be used

to seek out desired performance characteristics by identifying important genetic traits (117).

One of the recommended areas of research by the US National Research Council (NRC) is to use MCAs to detect disease organisms in embryos and semen (118). In the future, breeders in developing countries will probably attempt to improve breeding lines by importing frozen embryos and semen. If so, it will be important to certify that a disease endemic to one part of the world does not travel to another part via these import products. To prevent such occurrences, MCAs could be employed to detect viral and bacterial contaminants. For example, it is possible that the virus causing blue tongue could be present in the semen from a bull raised in the USA and that it could spread the disease to cows that are artifically inseminated with the contaminated semen. A specific MCA against the blue tongue virus would quickly determine whether suspect semen contains the virus.

Similar to applications in human health, MCAs may find use to detect and differentiate animal diseases, to analyze the antigenic make-up of pathogens, and as passive vaccines or anti-toxins. All these areas offer fertile possibilities for researchers in developing countries and elsewhere.

R+D in growth-promoting substances is potentially of high importance since it could result in increasing the world supply of high quality protein. At the same time, applications from this area of research will most probably take a long time to appear and only after extensive testing has been performed for safety and edibility. Therefore, a careful assessment will have to be made before such research is undertaken; an assessment which takes into full account the long time spans, expenses and difficulties involved before products are likely to be marketable.

Most of the R+D projects to be undertaken during the next five years in animal breeding are not likely to involve new biotechnology. Attention will most probably be given to increasing the success rate of using frozen embryos (now 20 - 50%), to seeking better methods of shipping frozen embryos and semen, to the identification and development of pedigrees, and to the establishment of gene banks to preserve valuable traits.

Biotechnology and Plant Agriculture

Plant products constitute an estimated 93% of the human diet; to satisfy human nutritional needs 3.75 billion tons of food were produced by the world's agriculture in 1980 of which 1.9 billion tons were edible dry matter (119). Agriculture is both the oldest and the largest of the world's industries and it will remain so for the foreseeable future. Dramatic gains in agriculture have been made in the last three decades largely as a result of the intensified use of land, fertilizers and pesticides and by planting improved crops developed through a variety of breeding programmes. In the years ahead, applications of new biotechnology techniques are likely to allow the high growth rate to continue. Two broad avenues of biotechnology R+D will here be investigated; the genetic improvement of plants and the development of efficient non-chemical pesticides.

Genetic Improvements of Plants.

Three general approaches may be taken to increase harvests; increase yields by using more productive plant strains, cut losses from pests and disease, and improve storage survival. Plant breeding incorporating new biotechnology techniques is likely to have important roles in each though most developments will occur in the long term.

The OTA's study on applied genetics presents a three-phase programme of plant breeding which incorporates some of the new biotechnology techniques (120). In phase I plant cells are isolated and kept alive in tissue culture. During phase II genetic engineering techniques are applied to alter the cells' genetic make-up. And in phase III the engineered cell is allowed to grow into whole plants.

Phase I: Plant Cell Cultures.

The basis of this technique rests on the ability of researchers to regenerate plants from a mass of disorganized tissue called callus. In the 1960s it was demonstrated that a single cultured cell could divide and form calluses; a little later, researchers were able to grow a complete and fertile plant from a single, isolated plant cell. At present, a large number of plants can be produced from a single callus. For example, one gram of starting carrot callus may produce 500 plants (119).

An important recent finding is that cells in a tissue culture, when allowed to develop into full plants, may differ markedly from one another though they all come from the same parent. This verification in cultured cells is called somaclonal variation (121). The genetic variability can be used to select out cells with desired characteristics by manipulating the media in which they grow. By using this approach it may, for example, be possible to select cells which exhibit marked tolerance to salts, metals, herbicides and extremes of soil acidity and alkalinity. However, much research remains to be done before this technique can be used to manufacture commercially valuable plants. A major limitation is that cells which are selected on the basis of a desired trait may not retain the trait when developed into a full plant, or undesirable traits may become apparent when the plant is grown.

Phase II: Genetic Engineering.

Due to the limited knowledge of plant molecular biology, little has been accomplished as to inserting foreign genes into plant cells. The reason hinges on the complexity of plants' genetic make-up. Genes in higher organisms are generally clustered into units called chromosomes. Gene function might be dependent on several factors: the sequence of genes on the chromosome; their spatial relationship; the mechanisms which "turn" the genes on and off, etc. For the foreign gene to become functional in a plant it must be first inserted into the nucleus of the plant cell without damaging the cell or its parts and, second, the gene must be incorporated correctly in the chromosome and its control mechanisms made to function. This series of steps is difficult to accomplish. However, a start has been made - foreign genes have been inserted into plants.

Two separate groups announced results from their work in this area at the 15th Miami Winter Symposium, January 1983. One group works for Monsanto while the other is a combined group from the Max Planck Institute in Cologne and the University of Ghent, Belgium. Both groups used nearly the same methods and the same vectors for transferring foreign genes into a plant. It has been known for a long time that the bacterium Agrobacter tumefaciens can cause tumours in plants by infiltrating part of its own genes into plant cells. Researchers were able to remove a plasmid (genetic material which is not part of the bacterial chromosome) from the bacterium, cut out the genes that code for tumour formation and replace them with foreign genes coding for resistance to the antibiotic kanamycin. The engineered bacterium was subsequently able to

infiltrate the foreign genes into a variety of plant cells, many of which were converted into healthy, intact plants highly resistant to kanamycin (122).

In another project researchers at Cetus Madison Corporation, the subsidiary of Cetus which concentrates on agricultural R+D, have been able to grow three generations of tobacco plants containing a foreign gene from yeast. Though the gene is not functional, Cetus believes the techniques developed as a consequence of this work will have far-reaching potential in adapting plants to commercial uses (123).

A promising line of research is underway at Michigan State University. Researchers there have found that a single gene codes for resistance of weeds to the herbicide atrazine. They are now working on developing herbicide-resistant crop plants by inserting the resistance gene into a target plant either through protoplast fusion or by direct introduction of the cloned genes into plant chloroplasts (124).

In addition to rDNA, the genetic make-up of plants can be altered by using protoplast fusion. In particular, interesting results have been achieved by fusing sexually incompatible species, for example, potato and tomato (125). The so-called pomato is not of immediate commercial value but the technique used to create it offers yet another means to genetically engineer plants. Severe limitations have to be overcome before protoplast fusion begins to impact on agriculture. Specifically, genetic information can be gained or lost during the fusion process in an uncontrolled manner. Therefore, the technique lacks the precision of rDNA.

Phase III: Plant Generation.

Severe problems have yet to be overcome in the regeneration of plants from single cells. The main problem is that the procedure has only been perfected in a few crops; the most important legume and grass crops have resisted regeneration efforts. Yet, a start has been made by three groups.

The two groups that succeeded in transferring the kanamycin-resistant gene to plant cells were able to grow full plants from the engineered cells. The variety of plants grown included petunias, sunflowers, tobacco and carrots.

A project of potential importance to the agro-industry is one being undertaken by Phytogen (USA) to produce improved cotton strains. Phytogen researchers are attempting to introduce a gene for herbicide resistance into cotton, preferably directly into the cotton callus (126). If this attempt is successful, stronger concentrations of herbicides could be used to control weed overgrowth. Details of the research are proprietary.

To conclude this part on genetic improvement of plants, brief comments will be made in reference to a potentially, extremely important research area: the incorporation of nitrogen fixation genes into plants.

Nitrogen is essential for plant growth and propagation. However, it must be available in a form which plants can use. Commonly, this is in a form found in chemically synthesized fertilizers. Important cereal crops almost totally dependent on fertilizers are wheat, corn, rice and forage grasses. A few plant species have developed symbiotic relationships with microorganisms which have the ability to combine atmospheric nitrogen with other elements such as carbon, hydrogen, and oxygen to form nitrogenous compounds useable by the plant in a process called nitrogen fixation (Nif). The symbiotic relationship is best exemplified by that existing between legumes and the soil bacteria Rhizobium where the latter is encapsulated in nodules found at the legume's roots. Several species of free-living bacteria also have Nif ability including the ubiquitous Klebsiella pneumoniae. At least 17 genes are involved in its Nif process.

The world demand for chemical fertilizer was an estimated 51.4 million tons in 1979 but the demand will grow to between 144 and 180 million tons by 2000 (127). Nif organisms fix approximately 175 million tons per year (128) and, of course, this quantity is not likely to increase unless new developments take place to increase plants' Nif capabilities.

Many research groups in both developed and developing countries are working on Nif - in terms of allocated funds for R+D, it may be the most important research area among the biological sciences. Efforts are under way to clarify the symbiotic relationships between plants and their Nif symbionts, to study the molecular regulation of Nif gene expression and to understand the energy requirements of the Nif process. Eventually, scientists hope to increase the role of Nif microorganisms by one, or more, of the following accomplishments: by genetically manipulating existing Nif systems in order to increase their efficiency; by transferring the Nif gene complex from bacteria

to plant so they can fix nitrogen directly from the air; and by growing large numbers of Nif microorganisms in fermenters and seeding them directly in the soil (soil inoculation).

The aim of Nif research is to allow farmers to lessen their dependence on chemical fertilizers. As energy costs have increased over the last decade, fertilizers have become excessively expensive thereby limiting their use in developing countries. And, in many cases, the unwise or excessive use of fertilizers has damaged soils by making them salty and has led to pollution of lakes, rivers and other water-shed.

Biotechnology and Pesticides.

The use of chemical pesticides has been beneficial to the world's population because the protection they afford to animal husbandry and plant agriculture has in part been responsible for the tremendous growth of agriculture in the last 30 years. At the same time, no one can ignore the largely unanticipated negative side effects that have accompanied pesticide use or that have become apparent with the passage of time. The hazards of pesticide use are as follows:

Persistence - the retention in eco-systems of chemicals where they cause damage to one or more organisms.

Lack of specificity - though a broad spectrum of activity may at times be desirable, at other times chemicals can kill beneficial species of insects and wildlife.

Resistance - with continued use of pesticides, many targeted weeds, insects and microorganisms have developed resistance against these agents. As resistance builds, larger and larger quantities of pesticides are required to achieve a sufficient level of lethal activity. As a result, more chemicals accumulate in the environment and the possibility of damage to non-target organisms increases. It has become a matter of some concern to agronomists that pests afflicting major crops are becoming extremely resistant to pesticides. For example, fears have arisen that the major pest of potato, the colorado potato beetle, and the major pest of turf grasses, the Japanese beetle, will soon become resistant to <u>all</u> conventional insecticides (129).

Carcinogenesis - chemicals may cause damage to organisms which may only become apparent in the long-term in the form of, for example, cancer.

Direct damage - the use of powerful chemical agents as pesticides requires

proper equipment and the operator possessing an adequate level of training and education. These prerequisites may not be readily available to workers, especially in the developing countries. Injury may as a result be caused to users of pesticides and to others accidentally exposed to their action.

Biotechnology offers several means of overcoming deleterious side effects by allowing farmers to adopt alternative strategies towards pest control. These include the use of biological control agents, attractants, and growth-affecting agents.

Biological Control Agents.

Microorganisms that infect insects are called entomopathogens and include bacteria, viruses and fungi. The best known of entomopathogens are bacteria of the genus Bacillus thuringiensis which have shown to be active against several insects. In particular, B. thuringiensis israelensis has a high degree of larvacidal activity against mosquitoes and blackflies while B. thuringiensis Sp-24 is active against the cotton leaf worm. Bacillus acts by secreting a toxin which is a stomach poison to larvae.

A few companies began producing commercial quantities of B. thuringiensis after the product was found safe by WHO and the US Environmental Protection Agency (EPA). The major shortcoming of the product is that it has a short residual effect necessitating frequent reapplications (130). The discovery, development and testing of entomopathogens has so far not involved new biotechnology. However, the situtaion is in the process of changing. Two R+D approaches can be discerned: (1) investigation centering on the toxins of entomopathogens, and (2) the transfer of genes controlling entomopathogenic activity from one microorganism to another.

Examples of each approach are as follows:

- A team led by A. Klier at Pasteur Institute have identified, isolated and cloned plasmids from B. thuringiensis carrying genes coding for crystal protein sporulation. Protein extracts from the clones have proven toxic to larvae (131). Another team at Kansas State University have isolated the gene coding for lepidoptera toxin and inserted the gene in E. coli and B. subtilis. The engineered organisms were subsequently able to synthesize the toxin (132).

- Researchers at the University of Georgia have been able to transfer plasmids containing genes coding for toxin production from producing strains of B. thuringiensis to non-producing Bacillus strains where the genes were expressed. Success was also achieved when a similar transfer was performed between B. thuringiensis and B. cereus (133).

Both approaches are in their formative stages, ie. the work being done is basic research. Though the toxin coding genes have been identified, isolated and transferred from B. thuringiensis to other organisms having industrial potential, it is much too early to know whether the toxins under investigation can actually be used on a large-scale in the field. Complex questions need to be answered before taking such a step can be contemplated. For example, the stability of the toxins has to be known; their effectiveness has to be measured; an assessment will have to be made of long-term environmental effects which large-scale field use of toxins may occasion, etc. Clearly, R+D may be undertaken in regard to entomopathogens with a good chance of results being achieved in the short-term. However, applications may be far off since products have to be tested and found safe to man and his environment. Endowing organisms now not considered to be entomopathogenic with such properties would pose an analogous situation; the safety and efficiency of the newly engineered organism will have to be measured and assessed before large-scale application is contemplated.

Attractants.

Female insects secrete minute amounts of chemicals called pheromones to attract males so mating can take place. Present research with pheromones is analogous to the situation vis-a-vis interferons a few years ago - some types of pheromones have been identified, occasionally a type has been used in experiments, but generally pheromones are not available to researchers because they only exist in minute quantities. The few experiments which have taken place suggest that proper placement of pheromones in the field act to confuse mating behavious to such an extent that insect procreation rates plummet and a concomitant high degree of insect control is achieved.

The extent of pheromone R+D taking place in regard to pheromones which utilize the new biotechnology techniques is not known. Yet, one may hypothesize that the chemical structures of some pheromones will soon become known and that immobilized enzymes will be used to convert readily available precursors to the complicated final product.

Growth-affecting agents.

Insects have complicated regulation systems controlled by the secretion of hormones which dictate when an insect enters or departs from its various growth phases. One can theorize that these hormones will be identified, investigated and their structures clarified within the next twenty years or so. Once this has been done, it should be possible to either isolate the genes which control their synthesis or to synthesize such genes, insert the genes into a proper host and produce large quantities of the hormone coded for. In this way, sufficient hormone would be available and, if found safe and efficient, applied in the field to deliberately disturb the growth of insects. Projects of this type would need a long-term commitment.

Storage survival.

Conventional breeding techniques in combination with tissue culturing have resulted in the raising of crops with enhanced rates of survival in transport and while stored. A particular noteworthy example is the tomato which has been bred to survive stresses of mechanical picking and truck transport. However, the genetic mechanisms which control traits which enhance survival are undoubtedly extremely complex and it will be many years before genetic engineering methods can be employed in this area.

Discussion

Agriculture is the most important of human industrial activities since its products affect immediately and directly the well-being of all people. Sufficient food is today being produced to provide each one of the Earth's population with 3,000 calories per day but as the rate of population growth is approximately 2% this satisfactory situation is bound to come to an end - by 2015 there will exist an estimated 8 billion people on Earth. Since the estimated 4 billion population in 1975 required 3.3 billion tons of food, 6.6 billion tons will be needed in 2015 (134). The increased demand can only be met by intensified production, by making new land available to farmers, and by developing new production possibilities, for example, in the marine environment.

The foregoing survey indicates that intensive activity is taking place related to increasing production in agriculture using new biotechnology techniques. The survey also shows that agricultural research now under way will mostly impact on production in the long term. Nevertheless, opportunities exist for R+D leading to short- and medium-term gains that can be profitably

undertaken by researchers in developing countries and at the future ICGEB. The importance which new biotechnology techniques will undoubtedly have for agriculture gives an added impetus for undertaking R+D in this area - R+D which should have both short-term and long-term components. The possibilities for R+D will for convenience be divided below into time frames but it must be recognized that no sharp delineations exist - developments in the short term will naturally give rise to further work in the medium and long term.

Short Term.

It is vital that third world researchers become thoroughly familiar with tissue culture. This technology affords researchers the ability to subject plant cells to manipulative techniques such as mutation, strain selection and process development and to be able to review results rather quickly. When a successful strain is recognized the whole plant can be grown and, in turn, its tissue used to seed other tissue cultures. One authority estimates that this cloning method is one million times faster than the traditional methods it replaced (135). Further, initial investment to set up a tissue culturing facility can often be quickly amortized. For example, in the US a commercial nursery may invest $ 50,000 to establish a tissue culture facility. This facility could breed a superior plant variety and produce 300,000 clones of this plant per month. Sale of clones would quickly pay off the initial investment (136).

A Kibbutz in Israel has demonstrated the commercial possibilities of establishing an industry based on tissue culture technology. A few years ago the Kibbutz Beit Haemek hired microbiologists and agro-scientists and invested in a facility for performing advanced tissue culturing in order to propagate ornamental and pathogen-free plants. After expanding their R+D activities (including setting up a linkage with the Weizmann Institute of Science) workers at the Kibbutz are developing plants resistant to herbicides, diseases and environmental hazards. Sales in 1982 from tissue-culture-derived products reached approximately $ 1 million, with 65% realized from exports (137).

Tissue culture technology can be particularly valuable to countries in the tropical regions that wish to exploit their botanical resources. The genetic variability in the form of germ cells or seeds (germplasm) offered by tropical forests and gardens present unending possibilities for utilization by agriculture and agro-industry. The unique genetic traits present in novel indigenous plants

can be used to improve crop strains in all parts of the world. Wild species have been utilized by agronomists for many years as a necessary source of genetic variation for introducing pest and disease resistance to crop plants. An illuminating example is the incorporation of a gene coding for resistance to striped rust; a fungal disease which afflicts barley. A suitable gene was found in an Ethiopian barley species and through the use of advanced breeding techniques was incorporated into US barley varieties, thereby saving the US farmers approximately $ 150 million per year (138).

An additional reason for undertaking R+D using the advanced breeding techniques is that it provides researchers with the tools to alleviate specific problems, such as plant disease, much faster than previously possibly. An example of how quickly agronomists can react to alleviate plant disease by using advanced plant breeding technologies is the effort, presently underway, to deal with the fungal disease, orange leaf rust, which threatens to destroy Mexico's major coffee-producing areas endangering an export worth $ 550 million per year. In response, a multi-national effort, utilizing the resources of Purdue University and the Coffee Rust Research Institute in Portugal, was started in 1983 primarily to develop a rust-resistant variety of coffee using tissue culture and other breeding techniques. By the time of this writing in the autumn of 1983, newly installed greenhouses are ready to begin turning out 200,000 cloned, rust-resistant plants every three weeks (139).

Opportunities for research pertaining to plant disease are numerous, for example, much can be done to develop palms resistant to the diseases cadang-cadang and root wilt; plants resistant to southern corn leaf blight; tobacco resistant to the bacterial disease wildfire, etc. Research pertaining to the transfer of genes between microorganisms and plants or from plant to plant is in its infancy. As was seen, only three successful attempts have been reported in the literature. However, the transfer or modification of single genes may offer short-term opportunities; for example, to incrementally improve steps in Nif or photosynthesis processes by replacing a gene coding for an enzyme with another coding for a more useful enzyme, and to alter the genetic code which controls amino acid synthesis in order to produce improved proteins. Of even greater immediacy is the requirement for more knowledge about vectors to be used to transfer genes. The vector now receiving the greatest attention is the Ti (for tumour-inducing) plasmid (140). The three groups that succeeded in transferring genes into plants did so with the Ti plasmid. It is carried by the plant pathogenic bacterium Agrobacterium tumefaciens which transfers part of the Ti plasmid into a plant where it causes crown gall tumour. Most

probably, a familiarity with the Ti plasmid will lead to short-term gains in knowledge on how to transfer functional genes to plants and for investigating the mechanism of gene regulation in plant cells.

Regeneration of whole plants from tissue cells is yet another fertile field of investigation with a short-term potential for pay-offs. An important limitation of this technique is that important crop plants have not been regenerated (including large-seeded legumes such as soy beans, beans and peas); neither have most forestry species (141). Researchers from developing countries may find it profitable to develop regeneration techniques for tropical plants now not being studied. If regeneration of an agriculturally important plant is accomplished, researchers may be able to employ somaclonal selection for follow-up work to select variants for possible use in crop improvement schemes, either at home or for export.

The possibility of gaining short-term pay-offs from tissue culture technology may be increased by the application of protoplast fusion. It was mentioned above that results from R+D utilizing this technique have tended to be haphazard since there is usually uncontrolled loss or gain of genetic material by the resultant hybrid plant. Nevertheless, familiarity with protoplast fusion techniques could allow researchers from developing countries to experiment with plants unique to their environments with a possibility of gaining results not possible elsewhere. In effect, due to the characteristics of this technique all who use it are near or at the same level of know-how. Advances that may result in commercially valuable products are just as likely to occur in a developing country as in a developed country. Furthermore, in a few areas of R+D countries located in the tropical zone could have certain advantages. For example, tropical countries may have unique plants and microorganisms that produce fragrance or aroma chemicals. Fragrance substances have a combined market value of approximately $ 2 billion per year (142). By using traditional breeding technologies, tissue culturing, protoplast fusion, somoclonal selection and regeneration it is probable that the number of plants producing different odoriferous substances can be increased, that the variety of pleasant odours can be expanded, and the quality of some fragrances can be improved. The makers of cosmetics, toiletries and detergents are continually seeking new and exotic fragrances so a ready market exists for products from plants engineered to produce new, pleasant odoriferous substances.

It is difficult to enumerate the many existing research opportunities in agriculture which will utilize new biotechnology techniques and are likely to result in short-term gains. In addition to those already discussed, the following possibilities using tissue culture and other plant breeding techniques may be mentioned (143);

- At the cellular level, to select mutants of the rice plant with high tolerances for salt and aluminium;
- To find and develop tobacco mutants having reduced photorespiration;
- To produce rice strains with high lysine and high protein contents;
- To develop rice strains resistant to sheath blight;
- To solve rice plant regeneration problems.

Medium-Term Projects.

Large areas of previously fertile agricultural lands have in the last two decades been degraded due to increasing soil salinity. The mechanisms whereby microbial and plant cells adapt to the stress of increased salt in the environment (or osmotic stress) is osmoregulation. This field is now being intensively studied in developed countries and it has become clear that genetic engineering technologies will play a great part in adapting plants to osmotic stress (144). Due to the many facets of osmoregulation, R+D in this area demands an integrated, multidisciplinary approach. At the minimum, scientists familiar with genetic regulation and transfer, with enzyme systems, and with cellular membrane functions will have to make up research teams. For reasons such as this, if a nation, or the future ICGEB, wishes to commence R+D in this area, it must be ready to make a major commitment in resources and time and not expect results leading to applications for at least five years.

The possibility of adapting Ti plasmids for use as vectors to carry foreign genes into plant has already been discussed. However, there exist several other pathogens that may be similarly utilized. Research in this area is lacking. Therefore, scientists in developing countries and at the ICGEB could be in a favourable position to investigate plant diseases peculiar to tropical and semi-tropical regions that are caused by disease agents having potential as vectors. Possible disease agents now under investigation are the cauliflower mosaic virus, the causative agent for oleander knot; and various phages that afflict bacterial pathogens of plants.

Suggestions for developing plant resistance to diseases, antibiotics, pesticides and saline have been made earlier in this chapter. It was pointed out that short-term results are most likely to occur when the factor to be modified is coded for by a single gene. It is more likely that most resistance factors are controlled by multi genes and will, therefore, be difficult to undertake with results to be achieved in the long term. R+D related to plant resistance against diseases, alkaline earth, anaerobic soil conditions (as a result of flooding), drought and changes in soil pH fall into this category.

In passing, it should be noted that researchers in developing countries may be in a particularly good position to perform R+D related to resistance for the following reasons: Much agriculture R+D in developed countries is funded by private industry or performed in industrial laboratories. This industry is largely made up of, or influenced by, giant chemical concerns and seed companies. Logically, it makes more commercial sense for these giants to develop better chemical agents to control plant disease and pests rather than invest in R+D leading to resistant plants. If this supposition is correct, it may be that R+D pertaining to plant resistance is underfunded, leaving a void that could be filled by researchers in developing countries.

To conclude the discussion on medium-term possibilities for R+D in agriculture, a mention should be made of an NRC recommendation pertaining to the use of MCAs in plant health (145). It was pointed out that limited means exist for identifying the large variety of pathogens causing disease among the world's important plant crops. Failure to identify disease agents has meant that their epidemiology is unknown. As a consequence, plant diseases are often not diagnosed in a timely manner leading to wrong and/or late treatment with resultant crop losses. High priority should, therefore, be given to developing MCAs against the following disease agents (see also Table II):

- Rice (rice dwarf, rice grassy stunt, rice ragged stunt, rice tungro);
- Maize (maize chlorotic leaf spot, maize rough dwarf, maize streak);
- Cassava (cassava mosaic virus);
- Citrus (citrus tristeza virus, Spiroplasma citri, Xanthomonas citri);
- Potato (potato viruses M, S, X and Y; potato leaf roll);
- Fruit trees (Prunus necrotic ring spot, apple mosaic, prune dwarf, tobacco streak, tomato ring spot, tobacco ring spot viruses).

Long-Term Projects.

Long-term projects holding promise for eventual practical results and that can be undertaken by researchers in developing countries and the ICGEB pertain to Nif, photosynthesis and plant growth. In each of these areas knowledge is lacking as to molecular mechanisms and the genes which code for processes.

Nif.

There are at least 17 genes which code for Nif in K. pneumoniae; the mechanism may be more complex in agriculturally important microorganisms such as Rhizobium. Severe problems need to be overcome: little is known of the mechanism of Nif; whether the whole complex of 17 genes (or more) is transferable, about Nif energy demands etc. Yet, as was suggested, there is a high probability of achieving results in the short term by emphasizing R+D on single genes of the Nif system. Such an effort would naturally lead to the long-range research suggested here.

Photosynthesis.

This process is used by green plants to gather energy from sunlight in order to combine carbon dioxide (CO_2) from the atmosphere with water from the soil to produce organic carbon compounds used for food and fibre. Oxygen is given off as a side product. Each phase of this complicated cycle is controlled by a host of genes whose locations and relationships are largely unknown. Possibilities exist for increasing photosynthetic efficiency by affecting metabolic pathways, modifying enzyme systems which catalyze chemical reactions, and by changing the physical structure of plants to make them respire more efficiently. None of these changes are likely to be accomplished within the next 15 years.

Plant growth.

The plant growth rates set the time of maturation, limit favourable time for harvests and affect the time between planting and harvest. Each plant has important stages in its life cycle; germination, seedling establishment, growing plant, flowering, and fruit production. Eventually, scientists may be able to identify the genes which control each stage and alter them to improve growth characteristics. For example, it may be possible to shorten the juvenile period of crops - the period during which no fruit appears. Because

little is known about the basic mechanisms controlling plant growth, meaningful genetic intervention into plant genomes for the purpose of affecting growth patterns will probably not occur within the next 20 years. It is possible that in the next 15 years tissue culture work and other advanced breeding techniques will offer better opportunities for affecting plant growth characteristics than do direct genetic interventions.

Conclusion

In the preceding pages, new biotechnology R+D in the pharmaceutical industry and in agriculture is reviewed. When industrialization of research results has occurred, or is likely to occur, it is so indicated. When possible, estimates are provided of the impacts which developments will have on society with special attention given to possible impacts in developing countries.

The review of developments in each industry is followed by an analysis of described events for the purposes of discerning R+D trends in developed countries and identifying presently neglected R+D possibilities. The results of analysis are used to identify R+D areas and projects having the potential for short-term pay-offs (in the form of applications useful to developing countries) and that can be undertaken by researchers in developing countries and at the ICGEB. At times, medium- and long-term R+D could lead naturally from projects launched because of their short-term potential. Examples are indicated.

As a result of the review and analysis, the following conclusions are reached:

1. The R+D area which offers the most possibilities for short-term pay-offs in terms of helping developing countries and in terms of marketing possibilities is R+D pertaining to vaccines. In particular rDNA techniques offer possibilities for designing and developing new or improved vaccines against many of the diseases afflicting man and animal in the third world (see Tables V, VII and VII). Short-term pay-offs from vaccine R+D could result for several reasons:

    (i) Advanced vaccine research is already going on in several places throughout the world so a relatively large pool of researchers exists in this area capable of performing directed research of interest to developing countries.

(ii) Recombinant DNA has become an accepted and widely used tool in biotechnology; most well-equipped bioscience laboratories could readily adapt this technique at a low cost.

(iii) If vaccines are improved through new genetic engineering techniques, it is possible a short-track approach could be taken to gain approval for their use by WHO and national governments.

(iv) New multi-valent vaccines made possible by incorporating several genes coding for different antigens (see page 11) could be used to prevent several diseases at one shot. The approved process for the multi-valent vaccines would no doubt be as costly and lengthy as other medicinals (see page 25) but it would still be somewhat less so than having to go through the approval process for each of the vaccines individually that are present in the multi-valent form.

(v) During the successful global programme to eradicate smallpox an infrastructure was established consisting of production facilities to produce smallpox vaccine and the technicians required to staff them. This infrastructure is still largely in place (though deteriorating) and it has been suggested that it could be reactivated in order to capitalize on results from present vaccine R+D. If so, a variety of vaccines could quite quickly be made available to health personnel in developing countries.

2. A second R+D area offering short-term pay-offs to developing countries is R+D pertaining to MCAs. The foregoing review should have made it clear MCAs have important roles in human, animal and plant health and that, therefore, MCA-related R+D can be the basis for an important industry. The reasons for establishing a strong MCA R+D capability include:

(i) The field is still in its infancy. Therefore, opportunity exists for catching up with researchers in developed nations.

(ii) The technology is relatively easy to master by properly trained and educated persons.

(iii) The initial capital investment required to establish advanced facilities including tissue culture equipment for MCA R+D is rather high when compared to other advanced genetic engineering technologies

such as rDNA yet it is not unreasonably high (see Table IX). However, scale-up would most likely present fewer problems and be much less costly in view of the extremely small quantities of MCAs that are usually required.

(iv) Researchers in a developing country can design, produce, and use specific MCAs in order to alleviate indigenous problems pertaining to human, animal or plant health. In doing so, researchers would accomplish two objectives; work to alleviate a national problem and add to the world's store of knowledge. Furthermore;

(v) Experience and know-how gained from producing one type of MCA can readily be transferred when production of other MCAs for use in related and unrelated R+D is contemplated.

(vi) Knowledge gained from producing MCAs for diagnostic purposes can easily be carried over to producing MCAs for therapeutic uses, if a decision to do so is made at a later time.

(vii) Reference is made to Tables VI, VII and VIII where specific infectious diseases afflicting humans, animals and plants are indicated. The columns "Requires improved diagnostic capability" indicate a number of diseases against which MCAs can be produced.

Establishing a capability for MCA R+D and manufacturing will ensure for developing countries many benefits and no detrimental side effects (except direct financial costs). An added attraction is that MCA manufacture and use are non-polluting and MCAs cannot cause harm to those who employ them.

3. The foregoing review also makes clear the important contributions that tissue culture and related new plant breeding technologies have made, and are likely to make, in improving agriculture throughout the world. Though tissue culture is not new and researchers in developing countries are aware of the technology and its uses (for example, 40 developing countries belong to the International Association for Plant Tissue Culture) the technology is not being routinely applied (146). Moreover, as was pointed out, tissue culture technology has been considerably augmented with the development of the new techniques of protoplast fusion, rDNA and somaclonal selection. For much the same reasons as given for MCAs, the reasons for developing nations to establish a strong capability in advanced plant breeding technology include:

(i) In some aspects (rDNA and protoplast fusion) the field is in its infancy so opportunity exists for catching up with research in the developed countries.

(ii) The techniques of the technology can be mastered relatively easily by trained and educated persons.

(iii) The capital investment required to establish an advanced plant breeding facility could be reasonable (see page 46) and past experience indicates that a high potential exists for the facility to quickly turn out products.

(iv) This technology is probably more "appropriate" to developing countries than any other advanced technology. Certainly, no other technology equals its potential for exploiting that greatest of national resources - biota.

(v) Familiarity with the advanced breeding techniques is useful across the spectrum of agricultural R+D.

(vi) A potentially useful cross-fertilization can take place between agricultural researchers using rDNA and other researchers also employing rDNA in non-related areas. A good potential exists for developments in one area being useful in another.

4. Except for vaccine R+D, rDNA offers less immediate possibilities for short-term pay-offs. However, this technique is potentially so important, no nation can ignore it. The foregoing review shows that several products have resulted from drug-related rDNA research; some are likely to have large markets, first in developed countries, then spreading to the third world. Due to the expensive, time-consuming effort required to develop and market drugs for human use, the application of genetic engineering in this area does not offer fertile opportunities to developing countries. However, other reasons exist for developing a strong capability in rDNA including:

(i) The technique is a powerful tool useful for elucidating gene structure and function. It cannot be ignored by anyone involved in either biology R+D or in a bioscience-based industry.

(ii) Recombinant DNA has by now become an accepted part of bioscience research - it is no longer considered novel. As such, it is relatively easily mastered by scientists and technicians.

(iii) As has been mentioned the capital investment required to establish or build up a strong rDNA capability is lower than is the case with MCAs and advanced plant breeding technology. Further, when compared with most other advanced technologies, the required investment is much less. However, scale-up of rDNA techniques to produce large-volume substances is usually very difficult and costly. The cost of rDNA research when compared to the application of that research may be 1 - 10.

(iv) The technique is widely applicable - it makes little difference if the technique is used in R+D relative to human, animal, plant or microbial cells. A strong rDNA programme has the potential for strengthening programmes across the spectrum of biology and biomedicine R+D.

(v) Researchers in developing countries have several possibilities for using rDNA in R+D with the potential for pay-offs in the short to medium term. For example, genetic interventions involving single genes offer intriguing possibilities.

5. In conclusion, it should be mentioned that a promising area of investigation pertaining to rDNA is the development of alternative hosts for molecular cloning. The commonly used bacterium, E. coli, has several disadvantages which can be circumvented by using alternate hosts (147). In particular, E. coli produces toxic substances at the same time it manufactures the desired protein thus causing difficulties with the purification process. Further, the desired protein is trapped within the E. coli cell necessitating an extra step of disintegrating all cells after reactions are complete. Finally, it is necessary to separate and purify the desired protein as a result of the aforementioned difficulties.

The bacterium Bacillus subtilis has several advantages over E. coli as a host. It does not produce toxins; it can be made to excrete products into the medium thereby facilitating their recovery; and it is already widely utilized in industry, particularly in the production of coenzymes and antibiotics.

According to C. Weissman, Biogen is already using a genetically engineered B. subtilis to produce human alpha 2 interferon. Since the interferon is secreted the yield can theoretically be increased by a factor of 10 over the method using E. coli. Actual yield has not reached this point as yet, possibly because of interfering enzymes (148).

Researchers are also looking at yeasts as possible hosts, particularly members of the Saccharomyces species. The advantages of yeasts over E. coli are that they are non-toxic (some are used as food); they are better adapted for growth in bioreactors; they secrete the product into the medium; and they offer opportunities to synthesize more complex substances than is possible in E. coli.

Research to find and develop alternate hosts for rDNA experiments is in its early stages - the opportunity is great for researchers in developing countries and elsewhere to significantly work in this field. Possibilities exist for discovering unique organisms in the environment of tropical and semi-tropical countries that could be better adapted for industrial purposes than any of the organisms now being used or being considered for use as alternate hosts.

TABLE I

ESTIMATED DEMAND FOR DRUGS BY THERAPEUTIC GROUPING

VALUE ($ 000 MILLION)

Therapeutic Group	1980	1990	2000
Antibiotics	8.25	18.00	40.50
Cardiovasculars	6.00	15.00	32.40
Anti-arthritics	3.75	10.50	24.30
Psycho-therapeutics	3.00	9.00	18.90
Analgesics	2.25	4.50	8.10
Cough and Cold Preps.	2.25	4.50	5.40
Diuretics	1.50	3.00	5.40
Steroids	1.50	4.50	10.80
Oestrogens	1.50	4.50	10.80
Cancer chemo-therapeutics	1.50	7.50	27.00
Others	43.50	69.00	86.40
TOTAL	75.00	150.00	270.00

TABLE II

RELATIVE IMPORTANCE OF DEVELOPED AND DEVELOPING COUNTRIES

AS DRUG MARKETS (1980-2000) ($ 000 MILLION)

	Developed Countries	Centrally Planned Economies*	Developing Countries
1980	52.50	12.15	10.35
1985	68.48	16.65	17.82
1990	100.19	21.76	28.05
1995	133.09	27.63	44.28
2000	143.60	33.63	92.77

* Excluding China

TABLE III

CHANGES IN THE LEADING 10 PHARMACEUTICAL MARKETS

TO THE YEAR 2000 ($ 000 MILLION)

	1979	1990	2000
United States	11,360	21,060	33,500
Japan	8,760	20,550	38,500
France	4,330	8,030	12,765
Italy	2,380	4,410	7,015
United Kingdom	2,000	3,700	5,895
Brazil	1,890	7,830	20,775
Spain	1,570	2,910	4,630
Argentina	1,400	3,835	7,475
Mexico	907	3,780	10,025
TOTAL WORLD	64,570	150,000	270,000

Source for Tables: OTA, 1983

TABLE IV

OTA ESTIMATES OF MONOCLONAL ANTIBODY MARKETS
(IN MILLIONS OF 1981 DOLLARS)

Application	1982 Market Size	1990 Market Size
1. Diagnostics		
In vitro diagnostic kits	20	300-500a,b
Immunohistochemical kits	nil	(40) 25
(examination of biopsies, smears, etc.)	nil	small-100[b,d]
In vivo diagnostics (primarily imaging)		
2. Therapeutics (includes radiolabeled and toxin-labeled reagents)	nil	500-1000[b,c]
3. Other		
Research products	small	10
Purification	small	10

a. High number indicates market for total kit, number in parenthesis indicates value of antibody alone for kit (includes patent licensing fees).

b. Variation depending on industry source, although the range has been corroborated by at least two sources.

c. This number could be much higher or lower depending on regulatory process.

d. Based on current pricing (1981 dollars) for diagnostic tests of the same type.

Source: OTA, 1983

TABLE V

POTENTIAL OF GENETIC ENGINEERING FOR THE IMPROVEMENT OF VACCINES

Disease	Category of Importance	Priority for Genetic Engineering in Vaccine Preparation
Viral diseases:		
Foot-and-mouth disease	1	High
Rinderpest	1	Low
Rabies	1	High
Vesicular stomatitis	2	Medium
Blue tongue	2	High
Hog cholera	2	Low
African swine fever	2	Unknown (high as a research tool)
African horse sickness	2	High
Venezuelan equine encephelomyelitis	2	Low
Malignant catarrhal fever	3	Low
Pulmonary adenomatosis	3	High
Aujeszky's disease	3	Medium
Viral diseases of poultry:		
Newcastle disease	1	Medium
Marek's disease	2	Low
Bacterial diseases:		
Tuberculosis	1	High
Brucellosis	1	Low
Clostridial infections	1	Low
Anthrax	2	Low
Pleuropneumonia	2	Low
Leptospirosis	3	Low

Source: Sir William Henderson, FRS (in Fishlock, 1982, p. 61)

TABLE VI

INFECTIOUS DISEASES OCCURRING IN PLANTS

Disease	Requires Improved Diagnostic Capability[a]	Work in Progress[b]
POTATO		
Bacterial		
Erwinia carotovora var. *atroseptica*		X
Pseudomonas solanacerum		X
Viral		
Andean potato latent		X
Potato leaf roll		X
Potato virus M		X
Potato virus S		X
Potato virus X		X
Potato virus Y		X
Tobacco mosaic		X
CITRUS (bacterial and spiroplasma)		
Citrus tristeza virus	X	X
Spiroplasma citri	X	X
Xanthomonas citri	X	X
FRUIT TREES (peach, plum, cherry, almond, apricot, apple)		
Viral		
Apple mosaic		X
Prune dwarf	X	X
Prunus necrotic ring spot		X
Tobacco ring spot	X	X
Tobacco streak	X	X
Tomato ring spot	X	X
COCONUT		
Cadang-cadang	X	
Lethal yellowing	X	
CASSAVA		
Cassava mosaic virus	X	X
SUGARCANE		
Ratoon stunt virus	X	X
Sugarcane mosaic virus	X	X
WHEAT, BARLEY, GRAINS		
Viral		
Barley stripe mosaic	X	
Barley yellow dwarf	X	
RICE		
Viral		
Rice dwarf	X	
Rice grassy stunt	X	
Rice ragged stunt	X	
Rice tungro	X	
MAIZE		
Viral		
Maize chlorotic leaf spot	X	
Maize rough dwarf	X	
Maize streak (Africa)	X	

[a] Relationships of pathogen host vector and environment not clearly understood.
[b] Can be controlled or reduced in severity.
Source: US Research Council, 1982.

TABLE VII

INFECTIOUS DISEASES OCCURRING IN HUMANS

Disease	Requires Improved Diagnostic Capability	Disease Control Possible[a]	Requires Vaccine or Better Vaccine
BACTERIAL			
Brucellosis	X		
Colibacillosis	X	X	
Leprosy	X		X
Leptospirosis	X	X	
Pseudomonoiasis	X		X
Salmonellosis	X	X	X
Syphilis	X		X
Tuberculosis	X	X	X
Venereal gonococcus	X		X
CHLAMYDIAL			
Trachoma	X	X	X
METAZOAN			
Ascariasis	X	X	X
Cysticercosis/hydatidosis	X	X	X
Filariasis	X	X	X
Schistosomiasis	X	X	X
PROTOZOAN			
Amebiasis	X	X	X
Giardiasis		X	X
Malaria	X	X	X
Toxoplasmosis	X	X	X
VIRAL			
Equine encephalitis	X		
Hepatitis	X	X	X
Herpes II venereal	X		X
Influenza	X	X	X
German measles	X		X
Rabies		X	X

[a] By vaccination, treatment, or by limiting spread.

Source: US National Research Council, 1982.

TABLE VIII

INFECTIOUS DISEASES OCCURRING IN ANIMALS

Disease	Requires Improved Diagnostic Capability	Disease Control Possible[a]	Requires Vaccine or Better Vaccine
BACTERIAL			
Atrophic rhinitis (swine)	X		
Brucellosis (bovine)	X	X	
Colibacillosis (swine, poultry)		X	
Leptospirosis (bovine, swine)	X	X	
Pleuropneumonia (swine)			X
Salmonellosis (swine, poultry)		X	
Tuberculosis (bovine)	X	X	X
METAZOAN			
Ascariasis		X	X
Cysticercosis/hydatidosis		X	X
Fascioliasis	X	X	X
Schistosomiasis		X	X
PROTOZOAN			
Babesiosis (bovine)	X	X	X
Coccidiosis (avian, ruminants)		X	X
Theileriosis		X	X
Trypanosomiasis	X	X	X
RICKETTSIAL			
Anaplasmosis (bovine)	X		X
VIRAL			
African swine fever	X		X
Aujeszky's disease (swine)		X	
Blue tongue (ruminants)	X		X
Bovine paralytic rabies	X	X	X
Bovine viral diarrhoea		X	X
Equine infectious anemia	X		X
Foot-and-mouth disease (swine, bovine)		X	X
Hog cholera		X	
Infectious bovine rhinotracheitis (bovine)	X	X	X
Low production syndrome (poultry)	X	X	X
Marek's disease (poultry)		X	X
Newcastle disease (poultry)		X	
Rinderpest (bovine)	X		X

[a] By vaccination, treatment, or by limiting spread.

Source: US National Research Council, 1982.

TABLE IX

COST ESTIMATE FOR AN MCA LABORATORY

Item	Estimated Cost (US Dollars)
CO_2 incubator	$ 3,000 - 5,000
Tissue culture sterile hood	3,000 - 8,000
Inverted microscope	1,000 - 8,000
Standard microscope	1,000 - 3,000
Water bath	300
Centrifuge	2,000 - 8,000
Autoclave	1,500 - 15,000
pH meter	400 - 1,000
Balance	1,200
Liquid nitrogen storage containers	3,000 - 6,000
Water distillation system	6,000 - 8,000
Mouse cages	3,000
Purchase of 50 mice for breeding stock	200
Maintain 150 mice for 12 months at $ 0.035 per day per mouse	1,890
Tissue culture flasks and plates	4,000
Tissue culture medium, buffers, antibiotics	2,000
Fetal calf serum	2,000
Plates for assay of antibodies	2,000
Additional glassware (beakers, bottles, pipettes)	1,000
TOTAL ESTIMATED COST	$41,000 - 75,000

The cost of furnishing supplies and equipment for a monoclonal antibody laboratory depends on the number of people employed, the sophistication of equipment utilized, and the pre-existing availability of required equipment and supplies. Assuming all required equipment and supplies would have to be purchased for the laboratory, the foregoing list is an estimate of intitial costs; labour costs and the laboratory facility itself are not included.

Estimated costs for optional equipment are: fluorescent microscope, $1,500; gamma scintillation counter, $15,000-25,000; and flow cytometer, $125,000-250,000. Total estimated cost with added equipment, $180,000-353,000.

The estimate for tissue culture supplies will vary according to the pace of activity in the laboratory. It takes $1,000 worth of supplies, on average, to carry the clones of hybridomas from a single fusion through to the first stage of culture and preservation. It takes approximately $300 per cell line to complete cloning and standardization.

Source: US National Research Council, op. cit., pp. 204-205.

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